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(54) Title: T1R3 A NOVEL TASTE RECEPTOR

(57) Abstract: The present invention relates to the discovery, identification and characterization of a receptor protein, referred to herein as T1R3, which is expressed in taste receptor cells and associated with the perception of bitter and sweet taste. The invention encompasses T1R3 nucleotides, host cell expression systems, T1R3 proteins, fusion protein, transgenic animals that express a T1R3 transgene, and recombinant "knock-out" animals that do not express T1R3. The invention further relates to methods for identifying modulators of the T1R3-mediated taste response and the use of such modulators to either inhibit or promote the perception of bitterness or sweetness. The modulators of T1R3 activity may be used as flavor enhancers in foods, beverages and pharmaceuticals.

T1R3 A NOVEL TASTE RECEPTOR

BACKGROUND

> The present invention relates to the discovery,
5 identification and characterization of a G protein coupled
receptor, referred to herein as T1R3, which is expressed in
taste receptor cells and associated with the perception of
sweet taste. The invention encompasses T1R3 nucleotides,
host cell expression systems, T1R3 proteins, fusion
10 proteins, polypeptides and peptides, antibodies to the T1R3
protein, transgenic animals that express a T1R3 transgene,
and recombinant "knock-out" animals that do not express
T1R3. The invention further relates to methods for
identifying modulators of the T1R3-mediated taste response
15 and the use of such modulators to either inhibit or promote
the perception of sweetness. The modulators of T1R3
activity may be used as flavor enhancers in foods,
b e v e r a g e s   a n d   p h a r m a c e u t i c a l s .

> The sense of taste plays a critical role in the life
20 and nutritional status of humans and other organisms.
Human taste perception may be categorized according to four
well-known and widely accepted descriptors, sweet, bitter,
salty and sour (corresponding to particular taste qualities
or modalities), and two more controversial qualities: fat
25 and amino acid taste. The ability to identify sweet-
tasting foodstuffs is particularly important as it provides
vertebrates with a means to seek out needed carbohydrates
with high nutritive value. The perception of bitter, on
the other hand, is important for its protective value,
30 enabling humans to avoid a plethora of potentially deadly
. plant alkaloids and other environmental toxins such as
ergotamine, atropine and strychnine. During the past few
years a number of molecular studies have identified
components of bitter-responsive transduction cascades, such
35 as α-gustducin (1, 2), Gγ13 (3) and the T2R/TRB receptors
(4-6). However, the components of sweet taste transduction
have not been identified so definitively (7, 8), and the

elusive sweet-responsive receptors have neither been cloned nor physically characterized.

Based on biochemical and electrophysiological studies of taste cells the following two models for sweet
5 transduction have been proposed and are widely accepted (7, 8). First, a GPCR-G_s-cAMP pathway - sugars are thought to bind to and activate one or more G protein coupled receptors (GPCRs) linked to G_s; receptor-activated $G\alpha_s$ activates adenylyl cyclase (AC) to generate cAMP; cAMP
10 activates protein kinase A which phosphorylates a basolateral K^+ channel, leading to closure of the channel, depolarization of the taste cell, voltage-dependent Ca^{++} influx and neurotransmitter release. Second, a GPCR-G_q/$G\beta\gamma$-IP_3 pathway - artificial sweeteners presumably bind to and
15 activate one or more GPCRs coupled to PLCβ2 by either the α subunit of G_q or by $G\beta\gamma$ subunits; activated $G\alpha_q$ or released $G\beta\gamma$ activates PLCβ2 to generate inositol trisphosphate (IP_3) and diacyl glycerol (DAG); IP_3 and DAG elicit Ca^{++} release from internal stores, leading to
20 depolarization of the taste cell and neurotransmitter release. Progress in this field has been limited by the inability to clone sweet-responsive receptors.

Genetic studies in mice have identified two loci, sac (determines behavioral and electrophysiological
25 responsiveness to saccharin, sucrose and other sweeteners) and dpa (determines responsiveness to D-phenylalanine), that provide major contributions to differences between sweet-sensitive and sweet-insensitive strains of mice (9-12). Sac has been mapped to the distal end of mouse
30 chromosome 4, and dpa mapped to the proximal portion of mouse chromosome 4 (13-16). The orphan taste receptor T1R1 was tentatively mapped to the distal region of chromosome 4, hence, it was proposed as a candidate for sac (17). However, detailed analysis of the recombination frequency
35 between T1R1 and markers close to sac in F2 mice indicates that T1R1 is rather distant from sac (~5 cM away according

2

to genetic data of Li et al (16); and more than a million
base pairs away from D18346, the marker closest to sac.
Another orphan taste receptor, T1R2, also maps to mouse
chromosome 4, however, it is even further away from
5   D18346/sac than is T1R1.

To thoroughly understand the molecular mechanisms
underlying taste sensation, it is important to identify
each molecular component in the taste signal transduction
10   pathways. The present invention relates to the cloning of
a G protein coupled receptor, T1R3, that is believed to be
involved in taste transduction and may be involved in the
changes in taste cell responses associated with sweet taste
perception.

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SUMMARY OF THE INVENTION

The present invention relates to the discovery,
identification and characterization of a novel G protein
coupled receptor referred to hereafter as T1R3, that
20   participates in the taste signal transduction pathway.
T1R3 is a receptor protein with a high degree of structural
similarity to the family 3 G protein coupled receptors
(herein after GPCR). As demonstrated by Northern Blot
analysis, expression of the T1R3 transcript is tightly
25   regulated, with the highest level of gene expression found
in taste tissue. In situ hybridization indicates that T1R3
is selectively expressed in taste receptor cells, but is
absent from the surrounding lingual epithelium, muscle or
connective tissue. Moreover, T1R3 is highly expressed in
30   taste buds from fungiform, foliate and circumvallate
papillae.

The present invention encompasses T1R3 nucleotides, host
cells expressing such nucleotides and the expression
products of such nucleotides. The invention encompasses
35   T1R3 protein, T1R3 fusion proteins, antibodies to the T1R3

3

receptor protein and transgenic animals that express a T1R3 transgene or recombinant knock-out animals that do not express the T1R3 protein.

Further, the present invention also relates to
5   screening methods that utilize the T1R3 gene and/or T1R3 gene products as targets for the identification of compounds which modulate, i.e., act as agonists or antagonists, of T1R3 activity and/or expression. Compounds which stimulate taste responses similar to those of sweet
10 tastants can be used as additives to act as flavor enhancers in foods, beverages or pharmaceuticals by increasing the perception of sweet taste. Compounds which inhibit the activity of the T1R3 receptor may be used to block the perception of sweetness.

15 The invention is based, in part, on the discovery of a GPCR expressed at high levels in taste receptor cells. In taste transduction, sweet compounds are thought to act via a second messenger cascade utilizing PLCß2 and IP_3. Co-localization of α-gustducin, $PLC\beta_2$, Gß3 and Gγ13 and T1R3 to
20 one subset of taste receptor cells indicates that they may function in the same transduction pathway.

DEFINITIONS

As used herein, italicizing the name of T1R3 shall
25 indicate the T1R3 gene, TIR3 DNA, cDNA, or RNA, in contrast to its encoded protein product which is indicated by the name of T1R3 in the absence of italicizing. For example, "T1R3" shall mean the T1R3 gene, TIR3 DNA, cDNA, or RNA whereas "T1R3" shall indicate the protein product of the
30 T1R3 gene.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A. Synteny between human 1p36.33 and mouse

4

4pter chromosomal regions near the mouse <u>Sac</u> locus. Shaded
circles indicate the approximate location of the predicted
start codons for each gene; arrows indicate the full span
of each gene including both introns and exons; arrowheads
5   indicate the approximate location of each polyadenylation
signal.   Genes indicated by lowercase letters were
predicted by Genscan and named according to their closest
homolog.   Genes indicated by capital letters (T1R3 and
DVL1) were experimentally identified and verified.   The
10   mouse marker D18346 indicated is closely linked to the <u>Sac</u>
locus and lies within the predicted pseudouridine synthase-
like gene.   The region displayed corresponds to ~45,000 bp;
the bottom scale marker indicates kilobases (K).

FIGURE 1B.   The nucleotide and predicted amino acid
15   sequences of human <u>T1R3</u>.   The ends of the introns are
indicated in highlighted lower case letters.

FIGURE 1C.   Predicted secondary structure of human
T1R3.   T1R3 is predicted to have seven transmembrane
helices and a large N-terminal domain.   Placement of the
20   transmembrane segments was according to the TMpred program.
Placement of the dimerization and ligand binding domain,
and the cysteine-rich domain were based on the mGluR1
receptor and other family 3 GPCRs (19).

FIGURE 2A.   Distribution of <u>T1R3</u> mRNA in mouse tissues
25   and mouse taste cells.   Autoradiogram of a Northern blot
hybridized with mouse <u>T1R3</u> cDNA.   Each lane contained 25 μg
of total RNA isolated from the following mouse tissues:
circumvallate and foliate papillae-enriched lingual tissue
(Taste), lingual tissue devoid of taste buds (Non-Taste),
30   brain, retina, olfactory epithelium (Olf Epi), stomach,
small intestine (Small Int), thymus, heart, lung, spleen,
skeletal muscle (Ske Mus), liver, kidney, uterus and
testis.   A 7.2 kb transcript was detected only in the taste
tissue, and a slightly larger transcript was detected in
35   testis.   The blot was exposed to X-ray film for three days.
The same blot was stripped and reprobed with a β-actin cDNA

5

(lower panel) and exposed for one day. The size of the RNA marker (in kilobases) is indicated in the right margin.

FIGURE 2B. The genomic sequence of the Sac region from mouse was used as a query to search the mouse

5   expressed sequence tag (est) database. Matches to the est database are shown in solid red and indicate exons; gaps in a particular est match are shown by black hashed lines and indicate an intron. The clustered nature of the est matches demarcates the extent of each of the genes within

10 this region. The near absence of ests at the position of T1R3 is consistent with the highly restricted pattern of expression seen in Figure 2a.

FIGURE 3A. T1R3 expression in taste receptor cells. Photomicrographs of frozen sections of mouse taste papillae

15 hybridized with ^{33}P-labelled antisense RNA probes for T1R3 and α-gustducin. Bright-field images of circumvallate (a), foliate (b), and fungiform (c) papillae hybridized to the antisense T1R3 probe demonstrate taste bud-specific expression of T1R3. Control bright-field images of

20 circumvallate (e), foliate (f), and fungiform papillae (g) hybridized to the sense T1R3 probe showed no nonspecific binding. The level of expression and broad distribution of T1R3 expression in taste buds was comparable to that of α-gustducin as shown in the bright field image of

25 circumvallate papilla hybridized to antisense α-gustducin probe (d). The control bright field image of circumvallate papilla hybridized to the sense α-gustducin probe (h) showed no nonspecific binding.

FIGURE 3B. Profiling the pattern of expression of

30 T1R3, α-gustducin, Gγ13 and PLCβ2 in taste tissue and taste cells. Left panel: Southern hybridization to RT-PCR products from murine taste tissue (T) and control non-taste lingual tissue (N). 3'-region probes from T1R3, α-gustducin (Gust), Gγ13, PLCβ2 and glyceraldehyde 3-

35 phosphate dehydrogenase (G3PDH) were used to probe the blots. Note that T1R3, α-gustducin, Gγ13 and PLCβ2 were

6

all expressed in taste tissue, but not in non-taste tissue. Right panel: Southern hybridization to RT-PCR products from 24 individually amplified taste receptor cells. 19 cells were GFP-positive (+), 5 cells were GFP-negative (-).

5   Expression of α-gustducin, Gγ13 and PLCβ2 was fully coincident. Expression of T1R3 overlapped partially with that of α-gustducin, Gγ13 and PLCβ2. G3PDH served as a positive control to demonstrate successful amplification of products.

10   FIGURE 4.   Co-localization of T1R3 PLCß2 and α-gustducin in taste receptor cells of human circumvallate papillae. (a, c) Longitudinal sections from human circumvallate papillae were labeled with rabbit antisera directed against a C-terminal peptide of human T1R3, along

15   with a Cy3-conjugated anti-rabbit secondary antibody. (b) T1R3 immunoreactivity in longitudinal sections from human papillae was blocked by pre-incubation of the T1R3 antibody with the cognate peptide.   (d) A longitutidinal section adjacent to that in sections of human fungiform papillae

20   double immunostained for T1R3 (h) and α-gustducin (i). The overlay of the two images is shown in (j). Magnification was 200X (a-d) or 400X (e-j).

FIGURE 5A. mT1R3 allelic differences. mT1R3 allelic differences between eight inbred mouse strains. All non-

25   taster strains showed identical sequences and were grouped in one row. In the bottom row the amino acid immediately before the position number is always from the non-tasters, while the amino acid immediately before the position number is from whichever tasters differed at that position from

30   the non-tasters.   The two columns in bold represent positions where all tasters differed from non-tasters and where the differences in nucleotide sequence result in amino acid substitutions. Nucleotide differences that do not alter the encoded amino acid are indicated as_s:

35   silent.   Nucleotide differences within introns are indicated as i: intron.

7

FIGURE 5B. Genealogy of the inbred strains of mice analyzed in (a). The year in which the strains were developed is indicated between brackets following the stain name. The laboratories in which these mice were
5   established are indicated.

FIGURE 6. The amino acid sequence of mouse T1R3 is aligned with that of two other rat taste receptors (rT1R1 and rT1R2), the murine extracellular calcium sensing (mECaSR) and the metabotropic glutamate type 1 (mGluR1)
10   receptors. Regions of identity among all five receptors are indicated by white letters on black; regions where one or more of these receptors share identity with T1R3 are indicated by black letters on gray. Boxes with dashed lines indicate regions predicted to be involved in
15   dimerization (based upon the solved structure for the amino terminal domain of mGluR1); filled circles indicate predicted ligand binding residues based on mGluR1; blue lines linking cysteine residues indicate predicted intermolecular disulfide bridges based on mGluR1. Amino
20   acid sequences noted above the alignment indicate polymorphisms that are found in all strains of nontaster mice. The predicted N-linked glycosylation site conserved in all five receptors is indicated by a black squiggle; the predicted N-linked glycosylation site specific to T1R3 in
25   nontaster strains of mice is indicated by the red squiggle.

FIGURE 7. The predicted three dimensional structure of the amino-terminal domain (ATD) of T1R3 modeled on that of mGluR1 (19) using the Modeller program. The model shows a homodimer of T1R3. (a) The view from the "top" of the
30   dimer looking down from the extracellular space toward the membrane. (b) The T1R3 dimer viewed from the side. In this view the transmembrane region (not displayed) would attach to the bottom of the dimer. (c) The T1R3 dimer is viewed from the side as in (b), except the two dimers have
35   been spread apart (indicated by the double headed arrow) to reveal the contact surface. A space-filling representation

(colored red) of three glycosyl moieties (N-acetyl-galactose-N-acetyl-galactose-Mannose) has been added at the novel predicted site of glycosylation of non-taster mT1R3. Note that the addition of even three sugar moieties at this

5   site is sterically incompatible with dimerization. Regions of T1R3 corresponding to those of mGluR1 involved in dimerization are shown by space filling amino acids. The four different segments that form the predicted dimerization surface are color-coded in the same way as are

10   the dashed boxes in Figure 5. The portions of the two molecules outside of the dimerization region are represented by a backbone tracing. The two polymorphic amino acid residues of T1R3 that differ in taster vs. non-taster strains of mice are within the predicted

15   dimerization interface nearest the amino terminus (colored light blue). The additional N-glycosylation site at aa58 unique to the non-taster form of T1R3 is indicated in each panel by the straight arrows.

20   DETAILED DESCRIPTION OF THE INVENTION

    T1R3 is a novel receptor that participates in receptor-mediated taste signal transduction and belongs to the family 3 G protein coupled receptors. The present invention encompasses T1R3 nucleotides, T1R3 proteins and

25   peptides, as well as antibodies to the T1R3 protein. The invention also relates to host cells and animals genetically engineered to express the T1R3 receptor or to inhibit or "knock-out" expression of the animal's endogenous T1R3.

30   The invention further provides screening assays designed for the identification of modulators, such as agonists and antagonists, of T1R3 activity. The use of host cells that naturally express T1R3 or genetically engineered host cells and/or animals offers an advantage in

35   that such systems allow the identification of compounds that affect the signal transduced by the T1R3 receptor protein.

9

Various aspects of the invention are described in greater detail in the subsections below.

THE T1R3 GENE

The cDNA sequence and deduced amino acid sequence of human
5   T1R3 is shown in Figure 1B. The T1R3 nucleotide sequences of the invention include: (a) the DNA sequence shown in Figure 1B; (b) nucleotide sequences that encode the amino acid sequence shown in Figure 1B; (c) any nucleotide sequence that (i) hybridizes to the nucleotide sequence set forth in
10   (a) or (b) under stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65EC, and washing in 0.1xSSC/0.1% SDS at 68EC (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green
15   Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and (ii) encodes a functionally equivalent gene product; and (d) any nucleotide sequence that hybridizes to a DNA sequence that encodes the amino acid sequence shown in Figure 1B, under less stringent
20   conditions, such as moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42EC (Ausubel et al., 1989 supra), yet which still encodes a functionally equivalent T1R3 gene product. Functional equivalents of the T1R3 protein include naturally occurring T1R3 present in species
25   other than humans. The invention also includes degenerate variants of sequences (a) through (d). The invention also includes nucleic acid molecules, that may encode or act as T1R3 antisense molecules, useful, for example, in T1R3 gene regulation (for and/or as antisense primers in
30   amplification reactions of T1R3 gene nucleic acid sequences).

In addition to the T1R3 nucleotide sequences described above, homologs of the T1R3 gene present in other species can be identified and readily isolated, without
35   undue experimentation, by molecular biological techniques well known in the art. For example, cDNA libraries, or

10

genomic DNA libraries derived from the organism of interest can be screened by hybridization using the nucleotides described herein as hybridization or amplification probes.

The invention also encompasses nucleotide sequences
5   that encode mutant T1R3s, peptide fragments of the T1R3, truncated T1R3, and T1R3 fusion proteins. These include, but are not limited to nucleotide sequences encoding polypeptides or peptides corresponding to functional domains of T1R3, including but not limited to, the ATD
10   (amino terminal domain) that is believed to be involved in ligand binding and dimerization, the cysteine rich domain, and/or the transmembrane spanning domains of T1R3, or portions of these domains; truncated T1R3s in which one or two domains of T1R3 is deleted, e.g., a functional T1R3
15   lacking all or a portion of the ATD region. Nucleotides encoding fusion proteins may include but are not limited to full length T1R3, truncated T1R3 or peptide fragments of T1R3 fused to an unrelated protein or peptide such as an enzyme, fluorescent protein, luminescent protein, etc.,
20   which can be used as a marker.

Based on the model of T1R3's structure, it is predicted that T1R3 dimerizes to form a functional receptor. Thus, certain of these truncated or mutant T1R3 proteins may act as dominant-negative inhibitors of the
25   native T1R3 protein. T1R3 nucleotide sequences may be isolated using a variety of different methods known to those skilled in the art. For example, a cDNA library constructed using RNA from a tissue known to express T1R3 can be screened using a labeled T1R3 probe. Alternatively,
30   a genomic library may be screened to derive nucleic acid molecules encoding the T1R3 receptor protein. Further, T1R3 nucleic acid sequences may be derived by performing PCR using two oligonucleotide primers designed on the basis of the T1R3 nucleotide sequences disclosed herein. The
35   template for the reaction may be cDNA obtained by reverse transcription of mRNA prepared from cell lines or tissue

11

known to express T1R3.

The invention also encompasses (a) DNA vectors that contain any of the foregoing T1R3 sequences and/or their complements (i.e., antisense); (b) DNA expression vectors
5   that contain any of the foregoing T1R3 sequences operatively associated with a regulatory element that directs the expression of the T1R3 coding sequences; (c) genetically engineered host cells that contain any of the foregoing T1R3 sequences operatively associated with a
10 regulatory element that directs the expression of the T1R3 coding sequences in the host cell; and (d) transgenic mice or other organisms that contain any of the foregoing T1R3 sequences. As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters,
15 enhancers, operators and other elements known to those skilled in the art that drive and regulate expression.

T1R3 PROTEINS AND POLYPEPTIDES

T1R3 protein, polypeptides and peptide fragments, mutated, truncated or deleted forms of the T1R3 and/or T1R3 fusion
20 proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, the identification of other cellular gene products involved in the regulation of T1R3 mediated taste transduction, and the screening for compounds that can be used to modulate taste
25 perception such as novel sweetners and taste modifiers.

Figure 1B shows the deduced amino acid sequence of the human T1R3 protein. The T1R3 amino acid sequences of the invention include the amino acid sequence shown in Figure 1B. Further, T1R3s of other species are encompassed
30 by the invention. In fact, any T1R3 protein encoded by the T1R3 nucleotide sequences described in Section 5.1, above, is within the scope of the invention.

The invention also encompasses proteins that are functionally equivalent to the T1R3 encoded by the
35 nucleotide sequences described in Section 5.1, as judged by

12

any of a number of criteria, including but not limited to
the ability of a sweet tastant to activate T1R3 in a taste
receptor cell, leading to transmitter release from the
taste receptor cell into the synapse and activation of an
5   afferent nerve. Such functionally equivalent T1R3 proteins
include but are not limited to proteins having additions or
substitutions of amino acid residues within the amino acid
sequence encoded by the _T1R3_ nucleotide sequences
described, above, in Section 5.1, but which result in a
10   silent change, thus producing a functionally equivalent
gene product.

Peptides corresponding to one or more domains of T1R3
(_e.g._, amino terminal domain, the cysteine rich domain
and/or the transmembrane spanning domains), truncated or
15   deleted T1R3s (_e.g._, T1R3 in which the amino terminal
domain, the cysteine rich domain and/or the transmembrane
spanning domains is deleted) as well as fusion proteins in
which the full length T1R3, a T1R3 peptide or a truncated
T1R3 is fused to an unrelated protein are also within the
20   scope of the invention and can be designed on the basis of
the _T1R3_ nucleotide and T1R3 amino acid sequences disclosed
herein. Such fusion proteins include fusions to an enzyme,
fluorescent protein, or luminescent protein which provide
a marker function.

25   While the T1R3 polypeptides and peptides can be
chemically synthesized (_e.g._, see Creighton, 1983,
Proteins: Structures and Molecular Principles, W.H. Freeman
& Co., N.Y.), large polypeptides derived from T1R3 and the
full length T1R3 itself may be advantageously produced by
30   recombinant DNA technology using techniques well known in
the art for expressing a nucleic acid containing _T1R3_ gene
sequences and/or coding sequences. Such methods can be
used to construct expression vectors containing the _T1R3_
nucleotide sequences described in Section 5.1 and
35   appropriate transcriptional and translational control
signals. These methods include, for example, _in vitro_

13

recombinant DNA techniques, synthetic techniques, and
<u>in vivo</u> genetic recombination. (See, for example, the
techniques described in Sambrook et al., 1989, <u>supra</u>, and
Ausubel et al., 1989, <u>supra</u>).

5   A variety of host-expression vector systems may be util
ized to express the <u>T1R3</u> nucleotide sequences of the
invention. Where the T1R3 peptide or polypeptide is
expressed as a soluble derivative (<u>e.g.</u>, peptides
corresponding to the amino terminal domain the cysteine
10   rich domain and/or the transmembrane spanning domain) and
is not secreted, the peptide or polypeptide can be
recovered from the host cell. Alternatively, where the
T1R3 peptide or polypeptide is secreted the peptide or
polypeptides may be recovered from the culture media.
15   However, the expression systems also include engineered
host cells that express T1R3 or functional equivalents,
anchored in the cell membrane. Purification or enrichment
of the T1R3 from such expression systems can be
accomplished using appropriate detergents and lipid
20   micelles and methods well known to those skilled in the
art. Such engineered host cells themselves may be used in
situations where it is important not only to retain the
structural and functional characteristics of the T1R3, but
to assess biological activity, <u>i.e.,</u> in drug screening
25   assays.

         The expression systems that may be used for purposes
of the invention include but are not limited to
microorganisms such as bacteria transformed with
recombinant bacteriophage, plasmid or cosmid DNA expression
30   vectors containing <u>T1R3</u> nucleotide sequences; yeast
transformed with recombinant yeast expression vectors
containing <u>T1R3</u> nucleotide sequences or mammalian cell
systems harboring recombinant expression constructs
containing promoters derived from the genome of mammalian
35   cells or from mammalian viruses.

         Appropriate expression systems can be chosen to

14

ensure that the correct modification, processing and sub-
cellular localization of the T1R3 protein occurs. To this
end, eukaryotic host cells which possess the ability to
properly modify and process the T1R3 protein are preferred.

5   For long-term, high yield production of recombinant T1R3
protein, such as that desired for development of cell lines
for screening purposes, stable expression is preferred.
Rather than using expression vectors which contain origins
of replication, host cells can be transformed with DNA

10   controlled by appropriate expression control elements and
a selectable marker gene, i.e., tk, hgprt, dhfr, neo, and
hygro gene, to name a few. Following the introduction of
the foreign DNA, engineered cells may be allowed to grow
for 1-2 days in enriched media, and then switched to a

15   selective media. Such engineered cell lines may be
particularly useful in screening and evaluation of
compounds that modulate the endogenous activity of the T1R3
gene product.

TRANSGENIC ANIMALS

20   The T1R3 gene products can also be expressed in
transgenic animals. Animals of any species, including, but
not limited to, mice, rats, rabbits, guinea pigs, pigs,
micro-pigs, goats, and non-human primates, e.g., baboons,
monkeys, and chimpanzees may be used to generate T1R3

25   transgenic animals.

Any technique known in the art may be used to
introduce the T1R3 transgene into animals to produce the
founder lines of transgenic animals. Such techniques
include, but are not limited to pronuclear microinjection

30   (Hoppe, P.C. and Wagner, T.E., 1989, U.S. Pat. No.
4,873,191); retrovirus mediated gene transfer into germ
lines (Van der Putten et al., 1985, Proc. Natl. Acad. Sci.
USA 82:6148-6152); gene targeting in embryonic stem cells
(Thompson et al., 1989, Cell, 56:313-321); electroporation

35   of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and
sperm-mediated gene transfer (Lavitrano et al., 1989, Cell
57:717-723); etc. For a review of such techniques, see

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Gordon, 1989, <u>Transgenic Animals, Intl. Rev. Cytol.</u> 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals
5   that carry the <u>T1R3</u> transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, <u>i.e.,</u> mosaic animals. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the
10   teaching of Lasko et al., (Lasko, M. et al., 1992, <u>Proc. Natl. Acad. Sci. USA</u> 89:6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is
15   desired that the <u>T1R3</u> transgene be integrated into the chromosomal site of the endogenous <u>T1R3</u> gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous <u>T1R3</u> gene are
20   designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous <u>T1R3</u> gene.

Once transgenic animals have been generated, the
25   expression of the recombinant <u>T1R3</u> gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of
30   the transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, <u>in situ</u> hybridization analysis, and RT-PCR. Samples of <u>T1R3</u> gene-expressing tissue may
35   also be evaluated immunocytochemically using antibodies specific for the <u>T1R3</u> transgene product.

16

ANTIBODIES TO T1R3 PROTEINS

　　　Antibodies that specifically recognize one or more epitopes of T1R3, or epitopes of conserved variants of T1R3, or peptide fragments of T1R3 are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

　　　The antibodies of the invention may be used, for example, in conjunction with compound screening schemes, as described, below, in Section 5.5, for the evaluation of the effect of test compounds on expression and/or activity of the T1R3 gene product.

　　　For production of antibodies, various host animals may be immunized by injection with a T1R3 protein, or T1R3 peptide. Such host animals may include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

　　　Polyclonal antibodies comprising heterogeneous populations of antibody molecules, may be derived from the sera of the immunized animals. Monoclonal antibodies may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-

17

cell hybridoma technique (Kosbor et al., 1983, _Immunology Today_ 4:72; Cole et al., 1983, _Proc. Natl. Acad. Sci._ USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, _Monoclonal Antibodies And Cancer Therapy_, Alan

5   R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclasses thereof. The hybridoma producing the mAb of this invention may be cultivated _in vitro_ or _in vivo._ Production of high titres of Mabs _in vivo_ makes this the

10 presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of

15 appropriate biological activity can be used (Morrison et al., 1984, _Proc. Nat'l. Acad. Sci._, 81:6851-6855; Neuberger et al., 1984, _Nature_, 312: 604-608; Takeda et al. 1985, Nature 314: 452-454). Alternatively, techniques developed for the production of humanized antibodies (U.S. Patent No.

20 5,585,089) or single chain antibodies (U.S. Patent No. 4,946,778 Bird, 1988, Science 242: 423-426; Huston et al., 1988, _Proc. Nat'l. Acad. Sci USA_, 85: 5879-5883; and Ward et al., 1989, _Nature_ 334: 544-546) may be used to produce antibodies that specifically recognize one or more epitopes

25 of T1R3.

SCREENING ASSAYS FOR DRUGS AND
OTHER CHEMICAL COMPOUNDS USEFUL
IN REGULATION OF TASTE PERCEPTION

The present invention relates to screening assay
30 systems designed to identify compounds or compositions that modulate T1R3 activity or _T1R3_ gene expression, and thus, may be useful for modulation of sweet taste perception.

In accordance with the invention, a cell-based assay system can be used to screen for compounds that modulate
35 the activity of the T1R3 and thereby, modulate the perception of sweetness. To this end, cells that

18

endogenously express T1R3 can be used to screen for compounds. Alternatively, cell lines, such as 293 cells, COS cells, CHO cells, fibroblasts, and the like, genetically engineered to express T1R3 can be used for

5   screening purposes. Preferably, host cells genetically engineered to express a functional T1R3 are those that respond to activation by sweet tastants, such as taste receptor cells. Further, ooyctes or liposomes engineered to express T1R3 may be used in assays developed to identify

10   modulators of T1R3 activity.

The present invention provides for methods for identifying a compound that induces the perception of a sweet taste (a "sweetness activator") comprising (i) contacting a cell expressing the T1R3 receptor with a test

15   compound and measuring the level of T1R3 activation; (ii) in a separate experiment, contacting a cell expressing the T1R3 receptor protein with a vehicle control and measuring the level of T1R3 activation where the conditions are essentially the same as in part (i), and then (iii)

20   comparing the level of activation of T1R3 measured in part (i) with the level of activation of T1R3 in part (ii), wherein an increased level of activated T1R3 in the presence of the test compound indicates that the test compound is a T1R3 activator.

25   The present invention also provides for methods for identifying a compound that inhibits the perception of a sweet taste (a "sweetness inhibitor") comprising (i) contacting a cell expressing the T1R3 receptor protein with a test compound in the presence of a sweet tastant and

30   measuring the level of T1R3 activation; (ii) in a separate experiment, contacting a cell expressing the T1R3 receptor protein with a sweet tastant and measuring the level of T1R3 activation, where the conditions are essentially the same as in part (i) and then (iii) comparing the level of

35   activation of T1R3 measured in part (i) with the level of activation of T1R3 in part (ii), wherein a decrease level

19

of activation of T1R3 in the presence of the test compound
indicates that the test compound is a T1R3 inhibitor.

A "sweet tastant", as defined herein, is a compound
or molecular complex that induces, in a subject, the
5   perception of a sweet taste.   In particular, a sweet
tastant is one which results in the activation of the T1R3
protein resulting in one or more of the following: (i) an
influx of Ca^{+2} into the cell; (ii) release of Ca^{+2} from
internal stores; (iii) activation of coupled G proteins
10   such as Gs and/or gustducin; (iv) activation of secon
messenger-regulating enzymes such as adenylyl cyclase
and/or phospholipase C. Examples of sweet tastants include
but are not limited to saccharin or sucrose, or other
sweetners.

15   In utilizing such cell systems, the cells expressing
the T1R3 receptor are exposed to a test compound or to
vehicle controls (e.g. , placebos). After exposure, the
cells can be assayed to measure the expression and/or
activity of components of the signal transduction pathway
20   of T1R3, or the activity of the signal transduction pathway
itself can be assayed.

The ability of a test molecule to modulate the
activity of T1R3 may be measured using standard biochemical
and physiological techniques. Responses such as activation
25   or suppression of catalytic activity, phosphorylation or
dephosphorylation of T1R3 and/or other proteins, activation
or modulation of second messenger production, changes in
cellular   ion   levels,   association,   dissociation   or
translocation of signaling molecules, or transcription or
30   translation of specific genes may be monitored. In non-
limiting   embodiments   of   the   invention,   changes   in
intracellular Ca^{2+} levels may be monitored by the
fluorescence of indicator dyes such as indo, fura, etc.
Additionally, changes in cAMP, cGMP, IP_3, and DAG levels may
35   be assayed.   In yet another embodiment, activation of
adenylyl cyclase, guanylyl cyclase, protein kinase A and

20

Ca^{2+} sensitive release of neurotransmitters may be measured to identify compounds that modulate T1R3 signal transduction. Further, changes in membrane potential resulting from modulation of the T1R3 channel protein can
5 be measured using a voltage clamp or patch recording methods. In yet another embodiment of the invention, a microphysiometer can be used to monitor cellular activity.

For example, after exposure to a test compound, cell lysates can be assayed for increased intracellular levels
10 of Ca^{2+} and activation of calcium dependent downstream messengers such as adenylyl cyclase, protein kinase A or cAMP. The ability of a test compound to increase intracellular levels of Ca^{2+}, activate protein kinase A or increase cAMP levels compared to those levels seen with
15 cells treated with a vehicle control, indicates that the test compound acts as an agonist (_i.e._, is a T1R3 activator) and induces signal transduction mediated by the T1R3 expressed by the host cell. The ability of a test compound to inhibit sweet tastant induced calcium influx,
20 inhibit protein kinase A or decrease cAMP levels compared to those levels seen with a vehicle control indicates that the test compound acts as an antagonist (_i.e._, is a T1R3 inhibitor) and inhibits signal transduction mediated by T1R3.

25   In a specific embodiment of the invention, levels of cAMP can be measured using constructs containing the cAMP responsive element linked to any of a variety of different reporter genes. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT),
30 luciferase, β-glucuronidase (GUS), growth hormone, or placental alkaline phosphatase (SEAP). Such constructs are introduced into cells expressing T1R3 thereby providing a recombinant cell useful for screening assays designed to identify modulators of T1R3 activity.

35   Following exposure of the cells to the test compound, the level of reporter gene expression may be quantitated to

21

determine the test compound's ability to regulate TIR3 activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture
5 supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177).
10 Such assays provide a simple, sensitive easily automatable detection system for pharmaceutical screening.

Additionally, to determine intracellular cAMP concentrations, a scintillation proximity assay (SPA) may be utilized (SPA kit is provided by Amersham Life Sciences,
15 Illinois). The assay utilizes ^{125}I-label cAMP, an anti-cAMP antibody, and a scintillant-incorporated microsphere coated with a secondary antibody. When brought into close proximity to the microsphere through the labeled cAMP-antibody complex, ^{125}I will excite the scintillant to emit
20 light. Unlabeled cAMP extracted from cells competes with the ^{125}I-labeled cAMP for binding to the antibody and thereby diminishes scintillation. The assay may be performed in 96-well plates to enable high-throughput screening and 96 well-based scintillation counting instruments such as those
25 manufactured by Wallac or Packard may be used for readout.

In yet another embodiment of the invention, levels of intracellular Ca^{2+} can be monitored using Ca^{2+} indication dyes, such as Fluo-3 and Fura-Red using methods such as those described in Komuro and Rakic, 1998, In: The Neuron
30 in Tissue Culture. L.W. Haymes, Ed. Wiley, New York.

Test activators which activate the activity of T1R3, identified by any of the above methods, may be subjected to further testing to confirm their ability to induce a sweetness perception. Test inhibitors which inhibit the
35 activation of T1R3 by sweet tastants, identified by any of the above methods, may then be subjected to further testing

22

to confirm their inhibitory activity.   The ability of the
test compound to modulate the activity of the T1R3 receptor
may be evaluated by behavioral, physiologic, or _in vitro_
methods.

5   For example, a behavioral study may be performed
where a test animal may be offered the choice of consuming
a composition comprising the putative T1R3 activator and
the same composition without the added compound.   A
preference for the composition comprising a test compound,
10   indicated, for example, by greater consumption, would have
a positive correlation with activation of T1R3 activity.
Additionally, lack of preference by a test animal of food
containing a putative inhibitor of T1R3 in the presence of
a sweetner would have a positive correlation with the
15   identification of an sweetness inhibitor.

In addition to cell based assays, non-cell based
assay systems may be used to identify compounds that
interact with, _e.g._, bind to T1R3.   Such compounds may act
as antagonists or agonists of T1R3 activity and may be used
20   to regulate sweet taste perception.

To this end, soluble T1R3 may be recombinantly
expressed and utilized in non-cell based assays to identify
compounds that bind to T1R3.   The recombinantly expressed
T1R3 polypeptides or fusion proteins containing one or more
25   of the domains of T1R3 prepared as described in Section
5.2, _infra_, can be used in the non-cell based screening
assays.   For example, peptides corresponding to the amino
terminal domain that is believed to be involved in ligand
binding and dimerization, the cysteine rich domain and/or
30   the transmembrane spanning domains of T1R3, or fusion
proteins containing one or more of the domains of T1R3 can
be used in non-cell based assay systems to identify
compounds that bind to a portion of the T1R3; such
compounds may be useful to modulate the signal transduction
35   pathway of the T1R3.   In non-cell based assays the
recombinantly expressed T1R3 may be attached to a solid

23

substrate such as a test tube, microtitre well or a column, by means well known to those in the art (see Ausubel et al., supra). The test compounds are then assayed for their ability to bind to the T1R3.

5   The T1R3 protein may be one which has been fully or partially isolated from other molecules, or which may be present as part of a crude or semi-purified extract. As a non-limiting example, the T1R3 protein may be present in a preparation of taste receptor cell membranes. In
10 particular embodiments of the invention, such taste receptor cell membranes may be prepared as set forth in Ming, D. et al., 1998, Proc. Natl. Sci. U.S.A. 95:8933-8938, incorporated by reference herein. Specifically, bovine circumvallate papillae ("taste tissue", containing
15 taste receptor cells), may be hand dissected, frozen in liquid nitrogen, and stored at -80EC prior to use. The collected tissues may then be homogenized with a Polytron homogenizer (three cycles of 20 seconds each at 25,000 RPM) in a buffer containing 10 mM Tris at pH 7.5, 10% vol/vol
20 glycerol, 1 mM EDTA, 1 mM DTT, 10 µg/µl pepstatin A, 10 µg/µl leupeptin, 10 µg/µl aprotinin, and 100 µM 4-(2-amino ethyl) benzenesulfoyl fluoride hydrochloride. After particulate removal by centrifugation at 1,500 x g for 10 minutes, taste membranes may be collected by centrifugation
25 at 45,000 x g for 60 minutes. The pelleted membranes may then be rinsed twice, re-suspended in homogenization buffer lacking protease inhibitors, and further homogenized by 20 passages through a 25 gauge needle. Aliquots may then be either flash frozen or stored on ice until use. As another
30 non-limiting example, the taste receptor may be derived from recombinant clones (see Hoon, M.R. et al., 1999 Cell 96, 541-551).

Assays may also be designed to screen for compounds that regulate T1R3 expression at either the transcriptional
35 or translational level. In one embodiment, DNA encoding a reporter molecule can be linked to a regulatory element of

24

the T1R3 gene and used in appropriate intact cells, cell extracts or lysates to identify compounds that modulate T1R3 gene expression. Appropriate cells or cell extracts are prepared from any cell type that normally expresses the

5   T1R3 gene, thereby ensuring that the cell extracts contain the transcription factors required for in vitro or in vivo transcription. The screen can be used to identify compounds that modulate the expression of the reporter construct. In such screens, the level of reporter gene expression is

10 determined in the presence of the test compound and compared to the level of expression in the absence of the test compound.

To identify compounds that regulate T1R3 translation, cells or in vitro cell lysates containing T1R3 transcripts

15 may be tested for modulation of T1R3 mRNA translation. To assay for inhibitors of T1R3 translation, test compounds are assayed for their ability to modulate the translation of T1R3 mRNA in in vitro translation extracts.

In addition, compounds that regulate T1R3 activity

20 may be identified using animal models.   Behavioral, physiological, or biochemical methods may be used to determine whether T1R3 activation has occurred. Behavioral and physiological methods may be practiced in vivo. As an example of a behavioral measurement, the tendency of a test

25 animal to voluntarily ingest a composition, in the presence or absence of test activator, may be measured. If the test activator induces T1R3 activity in the animal, the animal may be expected to experience a sweet taste, which would encourage it to ingest more of the composition. If the

30 animal is given a choice of whether to consume a composition containing a sweet tastant only (which activates T1R3) or a composition containing a test inhibitor together with a sweet tastant, it would be expected to prefer to consume the composition containing

35 sweet tastant only. Thus, the relative preference demonstrated by the animal inversely correlates with the

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activation of the T1R3 receptor.

Physiological methods include nerve response studies, which may be performed using a nerve operably joined to a taste receptor cell containing tissue, _in vivo_ or _in vitro._
5   Since exposure to sweet tastant which results in T1R3 activation may result in an action potential in taste receptor cells that is then propagated through a peripheral nerve, measuring a nerve response to a sweet tastant is, _inter alia_, an indirect measurement of T1R3 activation. An
10   example of nerve response studies performed using the glossopharyngeal nerve are described in Ninomiya, Y., et al., 1997, _Am. J. Physiol. (London)_ 272:R1002-R1006.

The assays described above can identify compounds which modulate T1R3 activity. For example, compounds that
15   affect T1R3 activity include but are not limited to compounds that bind to the T1R3, and either activate signal transduction (agonists) or block activation (antagonists). Compounds that affect _T1R3_ gene activity (by affecting _T1R3_ gene expression, including molecules, _e.g.,_ proteins or
20   small organic molecules, that affect transcription or interfere with splicing events so that expression of the full length or the truncated form of the T1R3 can be modulated) can also be identified using the screens of the invention. However, it should be noted that the assays
25   described can also identify compounds that modulate T1R3 signal transduction _(e.g.,_ compounds which affect downstream signaling events, such as inhibitors or enhancers of G protein activities which participate in transducing the signal activated by tastants binding to
30   their receptor). The identification and use of such compounds which affect signaling events downstream of T1R3 and thus modulate effects of T1R3 on the perception of taste are within the scope of the invention.

The compounds which may be screened in accordance
35   with the invention include, but are not limited to, small organic or inorganic compounds, peptides, antibodies and

26

fragments thereof, and other organic compounds (e.g., peptidomimetics) that bind to T1R3 and either mimic the activity triggered by the natural tastant ligand (i.e., agonists) or inhibit the activity triggered by the natural

5 ligand (i.e., antagonists). Such compounds may be naturally occurring compounds such as those present in fermentation broths, cheeses, plants, and fungi, for example.

Compounds may include, but are not limited to, peptides such as, for example, soluble peptides, including but not

10 limited to members of random peptide libraries (see, e.g., Lam, K.S. et al., 1991, Nature 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86); and combinatorial chemistry-- derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but

15 not limited to, members of random or partially degenerate, directed phosphopeptide libraries; (see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti- idiotypic, chimeric or single chain antibodies, and FAb,

20 F(ab')$_2$ and FAb expression library fragments, and epitope binding fragments thereof), and small organic or inorganic molecules.

Other compounds which may be screened in accordance with the invention include but are not limited to small

25 organic molecules that affect the expression of the T1R3 gene or some other gene involved in the T1R3 signal transduction pathway (e.g., by interacting with the regulatory region or transcription factors involved in gene expression); or such compounds that affect the activity of

30 the T1R3 or the activity of some other intracellular factor involved in the T1R3 signal transduction pathway, such as, for example, a T1R3 associated G-protein.

COMPOSITIONS CONTAINING MODULATORS
OF T1R3 AND THEIR USES

35   The present invention provides for methods of inducing a sweet taste resulting from contacting a taste

27

tissue of a subject with a sweet tastant, comprising
administering to the subject an effective amount of a
T1R3 activator, such as a T1R3 activator identified by
measuring T1R3 activation as set forth in Section 5.5

5   supra.   The present invention also provides for methods
of inhibiting the sweet taste of a composition,
comprising incorporating, in the composition, an
effective amount of a T1R3 inhibitor. An "effective
amount" of the T1R3 inhibitor is an amount that

10   subjectively decreases the perception of sweet taste
and/or that is associated with a detectable decrease in
T1R3 activation as measured by one of the above assays.

     The present invention further provides for a method
of producing the perception of a sweet taste by a

15   subject, comprising administering, to the subject, a
composition comprising a compound that activates T1R3
activity such as a sweetness activator identified as set
forth in Section 5.5 supra. The composition may comprise
an amount of activator that is effective in producing a

20   taste recognized as sweet by a subject.

     Accordingly, the present invention provides for
compositions comprising sweetness activators and
sweetness inhibitors. Such compositions include any
substances which may come in contact with taste tissue of

25   a subject, including but not limited to foods, beverages,
pharmaceuticals, dental products, cosmetics, and wetable
glues used for envelopes and stamps.

     In one set of embodiments of the invention, T1R3
activators are utilized as food or beverage sweetners.

30   In such instances, the T1R3 activators of the invention
are incorporated into foods or beverages, thereby
enhancing the sweet flavor of the food or beverage
without increasing the carbohydrate content of the food.

     In another embodiment of the invention, a sweetness

35   activator is used to counteract the perception of

28

bitterness associated with a co-present bitter tastant.
In these embodiments, a composition of the invention
comprises a bitter tastant and a sweetness activator,
where the sweetness activator is present at a

5   concentration which inhibits bitter taste perception.
For example, when the concentration of bitter tastant in
the composition and the concentration of sweetness
activator in the composition are subjected to an assay as
disclosed in Section 5.1 supra.

10   The present invention may be used to improve the
taste of foods by increasing the perception of sweetness
or by decreasing or eliminating the aversive effects of
bitter tastants. If a bitter tastant is a food
preservative, the T1R3 activators of the invention may

15   permit or facilitate its incorporation into foods,
thereby improving food safety. For foods administered as
nutritional supplements, the incorporation of T1R3
activators of the invention may encourage ingestion,
thereby enhancing the effectiveness of these compositions

20   in providing nutrition or calories to a subject.

The T1R3 activators of the invention may be
incorporated into medical and/or dental compositions.
Certain compositions used in diagnostic procedures have
an unpleasant taste, such as contrast materials and local

25   oral anesthetics. The T1R3 activators of the invention
may be used to improve the comfort of subjects undergoing
such procedures by improving the taste of compositions.
In addition, the T1R3 activators of the invention may be
incorporated into pharmaceutical compositions, including

30   tablets and liquids, to improve their flavor and improve
patient compliance (particularly where the patient is a
child or a non-human animal).

The T1R3 activators of the invention may be
comprised in cosmetics to improve their taste features.

35   For example, but not by way of limitation, the T1R3
activators of the invention may be incorporated into face

29

creams and lipsticks. In addition, the T1R3 activators
of the invention may be incorporated into compositions
that are not traditional foods, beverages,
pharmaceuticals, or cosmetics, but which may contact
5   taste membranes. Examples include, but are not limited
to, soaps, shampoos, toothpaste, denture adhesive, glue
on the surfaces of stamps and envelopes, and toxic
compositions used in pest control (e.g., rat or cockroach
poison).
10

EXAMPLE: CLONING AND CHARACTERIZATION OF THE T1R3
GENE

The data presented below describes the identification
of a novel taste receptor, T1R3, as being Sac.   This
15   identification is based on the following observations.
T1R3 is the only GPCR present in a 1 million bp region of
human genomic DNA centered on the D18346 marker most
tightly linked to Sac.   Expression of T1R3 is narrowly
restricted and is highly expressed in a subset of taste
20   receptor cells. Expression of T1R3 in taste receptor cells
overlaps in large part with known and proposed elements of
sweet transduction pathways (i.e. α-gustducin, Gγ13. T1R3
is a family 3 GPCR with a large extracellular domain
sensitive to proteases (a known property of the sweet
25   receptor).   Most tellingly, a polymorphism in T1R3 was
identified that differentiated all taster strains of mice
from all non-taster strains: T1R3 from non-tasters is
predicted to contain an N-terminal glycosylation site that
based on modeling of T1R3's structure would be expected to
30   interfere with its dimerization.   Hence, not only is T1R3
identified as sac, but based on the model of T1R3 and this
polymorphic change it is also likely to be a sweet-
responsive (i.e. sweet-liganded) taste receptor.

35   GENE IDENTIFICATION
To identify the mouse gene (pseudouridine synthase-
like) containing the D18346 marker the D18346 sequence was

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used as a query sequence in a BlastN screen of the mouse
expressed sequence tag (est) database.   Each resulting
overlapping sequence match was used iteratively to extend
the sequence until the nearly full length gene was
5   determined.   The resulting contig was translated and the
predicted open reading frame was used as a query in a
TBlastN search of the High Throughput Genomic Sequence
(HTGS) database.   This search located a human BAC clone
AL139287 containing the human ortholog.   Genscan was used
10   to predict genes and exons in this clone.   BlastN or
TBlastN searches of either the NR or the est databases were
used to further define known or unknown genes in this and
other clones.   Each resulting predicted gene was used in
TBlastN or BlastN searches of the HTGS to find overlapping
15   BAC or PAC clones.   Each of the overlapping sequences was
used in BlastN searches of the HTGS to continue the build
of an unordered contig of the region.   The predicted genes
and exons that resulted from this search were used to
partially order over 1 million bases of genomic sequence
20   centered on the pseudouridine synthase-like gene containing
the D18346 marker.   Two human clones were found to contain
T1R3, the aforementioned AL139287 and AC026283.   The human
T1R3 gene was first predicted by Genscan and subsequently
confirmed by RT-PCR of human fungiform taste bud RNA and/or
25   screening of a human taste library.   In addition to the
above manipulations and searches we used an algorithm
(designed to recognize transmembrane spans in genomic
sequence) to search all of the human genomic clones on the
p arm of human chromosome 1 from 1pter to 1p33 (Sanger
30   Center chromosome 1 mapping project, FC and HW,
unpublished).   This screen predicted T1R3 as well as T1R1
and T1R2.   Human T1R3 lies within 20,000 bp of the D18346
marker and the pseudouridine synthase-like gene and is the
only predicted GPCR in this 1 million bp region.

35   The human predicted gene was then used in a TBlastN
screen of the Celera mouse fragment genomic database.   Each
matching fragment was used to fill gaps and further extend

31

the mouse T1R3 ortholog in repeated BlastN searches. The
following mouse fragments were used to build and refine the
mouse T1R3 genomic sequence: GA_49588987, GA_72283785,
GA_49904613, GA_50376636, GA_74432413, GA_70914196,
5   GA_62197520, GA_77291497, GA_74059038, GA_66556470,
GA_70030888, GA_50488116, GA_50689730, ` GA_72936925,
GA_72154490, GA_69808702. Genscan was used to predict the
mouse gene from the resulting genomic contig. The
predicted mouse T1R3 gene was confirmed by RT-PCR of mouse
10 taste bud RNA. Other genes from the human genomic region
centered on D18346 were used to search the Celera mouse
fragments database. The sequences from these searches were
used to build a mouse genomic contig of this region and
confirm the linkage of D18346 with T1R3 in the mouse genome
15 and the micro-synteny of the human and mouse genes in this
region. One gap in the genomic sequence, between the 5'-
end of T1R3 and the 3'-end of the glycolipid-transferase-
like gene was bridged by PCR and confirmed by sequence
analysis.

20   NORTHERN HYBRIDIZATION

Total RNAs were isolated from several mouse tissues
using the Trizol reagents, then 25 µg of each RNA was
electrophoresed per lane on a 1.5% agarose gel containing
6.7% formaldehyde. The samples were transferred and fixed
25 to a nylon membrane by UV irradiation. The blot was
prehybridized at 65 °C in 0.25 M sodium phosphate buffer (pH
7.2) containing 7% SDS and 40 µg/ml herring sperm DNA with
agitation for 5 hours; hybridization for 20 hours with the
^{32}P-radiolabeled mouse T1R3 probe was carried out in the
30 same solution. The membrane was washed twice at 65 °C in 20
mM sodium phosphate buffer (pH 7.2) containing 5% SDS for
40 minutes, twice at 65 °C in the same buffer containing 1%
SDS for 40 minutes, and once at 70 °C in 0.1 x SSC and 0.1%
SDS for 30 minutes. The blot was exposed to X-ray film for
35 3 days at ˉ80 °C with dual intensifying screens. The ^{32}P-
labeled T1R3 probe was generated by random nonamer priming

32

of a 1.34-kb cDNA fragment of murine T1R3 corresponding to
the 5'-end coding sequence using Exo(-) Klenow polymerease
in the presence of $(\alpha-^{32}P)$-dCTP.

IN SITU HYBRIDIZATION

5   33P-labeled RNA probes T1R3 (2.6 kb) and `α-gustducin (1
kb)] were used for in situ hybridization of frozen sections
(10 μm) of mouse lingual tissue. Hybridization and washing
were as described (2). Slides were coated with Kodak NTB-2
nuclear track emulsion and exposed at 4°C for 3 weeks and
10   then developed and fixed.

GENE EXPRESSION PROFILING

Single taste receptor cell RT-PCR products (5 μl) were
fractionated by size on a 1.6% agarose gel and transferred
onto a nylon membrane. The expression patterns of the
15   isolated cells were determined by Southern hybridization
with 3'-end cDNA probes for mouse T1R3, α-gustducin, Gγ13,
PLCβ2 and G3PDH. Blots were exposed for five hours at ⁻80
°C. Total RNAs from a single circumvallate papilla and a
similar-sized piece of non-gustatory epithelium were also
20   isolated, reverse transcribed, amplified and analyzed as
for the individual cells.

IMMUNOCYTOCHEMISTRY

Polyclonal antisera against a hemocyanin-conjugated T1R3
peptide (T1R3-A, aa 829-843) were raised in rabbits. The
25   PLC   β2   antibody   was   obtained   from   Santa-Cruz
Biotechnologies. Ten micron thick frozen sections of human
lingual tissue (previously fixed in 4% paraformaldehyde and
cryoprotected in 20% sucrose) were blocked in 3% BSA, 0.3%
Triton X-100, 2% goat serum and 0.1% Na Azide in PBS for 1
30   hour at room temperature and then incubated for 8 hours at
4 °C with purified antibody against α-gustducin, or
antiserum against T1R3 (1:800). The secondary antibodies
were Cy3-conjugated goat-anti-rabbit Ig for T1R3 and

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fluorescein-conjugated goat-anti-rabbit Ig for PLC β2. PLC
β2 and T1R3 immunoreactivities were blocked by
preincubation of the antisera with the corresponding
5   synthetic peptides at 10 µM and 20 µM, respectively.
Preimmune serum did not show any immunoreactivity. Some
sections were double-immunostained with T1R3 and PLC β2
antisera as described (46). Briefly, sections were
incubated sequentially with T1R3 antiserum, anti-rabbit-Ig-
10   Cy3 conjugate, normal anti-rabbit-Ig, PLCβ2 antibody and
finally with anti-rabbit-Ig-FITC conjugate with
intermittent washes between each step. Control sections
that were incubated with all of the above except PLCβ2
antibody did not show any fluorescence in the green
15   channel.

IDENTIFICATION OF SEQUENCE POLYMORPHISMS IN mT1R3

Based on the sequence of mouse T1R3 obtained from the
Celera mouse fragments database, oligonucleotide primers
20   were designed to amplify DNA encoding regions with open
reading frames. Total RNA isolated from taste papillae or
tail genomic DNA isolated from one taster (C57BL/6J) and
one non-taster (129/Svev) mouse strain each were used as
templates to amplify mouse T1R3 cDNA and genomic DNA using
25   RT-PCR and PCR, respectively. PCR products were sequenced
completely in an ABI 310 automated sequencer. Based on the
sequence obtained, four sets of oligonucleotide primers
were used to amplify the T1R3 regions where polymorphisms
were found between the two strains of mice. Genomic DNA
30   from mouse strains DBA/2, BALB/c, C3H/HeJ, SWR and FVB/N,
was used as template. The amplicons were purified and
directly sequenced. The genealogical tree of these strains
of mice was based on Hogan et al, (47) and the Jackson
laboratory web site (http://www.jax.org).

35   ## MODELING THE STRUCTURE OF T1R3

34

The amino terminal domains (ATDs) of mouse T1R3 and
mouse GluR1 were aligned using the ClustalW program (48).
The alignment was manually edited to generate an optimal
alignment based on structural and functional
5   considerations. Atomic coordinates of the mGluR1 ATD
crystal structure (19) were obtained from the protein
database and were used along with the alignment as the
source of spatial restraints for modeling. The
structural model of mouse T1R3 was generated using the
10   program MODELLER (49). The original images for Fig.7
were created using the programs Insight II and Weblab
Viewer (Molecular Simulations Inc.) and then imported
into Photoshop where the open view was created and the
labels were added.

15

RESULTS

MAPPING OF THE MURINE AND HUMAN HUMAN SAC REGIONS

The murine Sac gene is the primary determinant of
inter-strain preference responses to sucrose, saccharin,
20   acesulfame, dulcin, glycine and other sweeteners (9-12),
however, the molecular nature of the Sac gene product is
unknown. Taster vs. non-taster strains of mice display
differences in the electrophysiological responses of
their taste nerves to sweeteners and sweet amino acids,
25   arguing that Sac exerts its effect on the sweet pathway
at the periphery (14, 18). The most likely explanation
for these differences is an allelic difference in a gene
encoding a sweet-responsive taste transduction element
such as a receptor, G protein subunit, effector enzyme or
30   other member of the sweet signaling pathway. It had been
speculated that the Sac gene product modified a sweet-
responsive receptor (12), was itself a taste receptor
(17) or a G protein subunit (14). As a first step toward
identifying the nature of the Sac gene we generated a
35   contiguous map of the human genome in this region was
generated.

35

Starting with the mouse marker D18346 (16), which maps
most closely to the sac locus at 4pter, a novel mouse
gene from the est database was identified: D18346 is
found in the 3' untranslated region (UTR) of a novel
5   mouse gene with homology to pseudouridine synthase. At
the time this work was initiated the sequence of the
human genome was nearly complete (although only partially
assembled), while that of mouse was quite incomplete,
hence, finished human genomic sequences and unfinished
10   sequences from bacterial artificial chromosome (BAC) and
P1 artificial chromosome (PAC) clones known to map to
human chromosome 1pter - 1p36.33 (syntenic to mouse
4pter) was screened for the ortholog of the novel
pseudouridine synthase-like gene containing the D18346
15   marker. Using the TblastN program the high-throughput
human genomic sequence (HTGS) database (NCBI) was
searched to identify a PAC clone containing the human
ortholog of the pseudouridine synthase-like gene. By
repeated Blast searches of the human HTGS with portions
20   of the sequence from this and overlapping PAC and BAC
clones we were able to form a contiguous map ("contig")
of 6 overlapping BAC or PAC clones spanning approximately
one million bp of human genomic DNA sequence was found.

Using the Genscan gene prediction program we identified
25   the predicted exons and genes within this contig were
identified. Twenty three genes were predicted in this
region (Figure 1A), including "pseudouridine synthase-
like", "cleavage and polyadenylation-like", and
"glycolipid transfer-like"; a few genes within this
30   region had been previously identified and/or
experimentally verified by others (e.g. disheveled 1,
dvl1). The Celera mouse genomic database was searched to
identify the murine orthologs of the genes within this
region and pieced together the mouse contig (Figure 1A).

35   IDENTIFICATION OF A NOVEL RECEPTOR,
                      T1R3, WITHIN THE SAC REGION

                                  36

In the screen of the million bp of genomic DNA sequence in the Sac region, only one predicted GPCR gene was found. The gene, which was referred to as T1R3 (for taste receptor one, member three family), was of special

5   interest because the predicted protein it encodes is most similar to T1R1 and T1R2, two orphan GPCRs expressed in taste cells (17), and because, as will be shown below, it is expressed specifically in taste cells. Human T1R3 (hT1R3) is located about 20kb from the pseudouridine

10   synthase-like gene, the human ortholog of the mouse gene containing the D18346 marker (Figure 1A). If T1R3 is Sac, then its proximity to D18346 is consistent with the previously observed very low probability of crossovers between the marker and the Sac locus in F2 crosses and

15   congenic mice (16).

The intron/exon structure of the coding portion of the hT1R3 gene was predicted by Genscan to span 4 kb and contain 7 exons (Figure 1B). To confirm and refine the inferred amino acid sequence of the predicted hT1R3 protein

20   we cloned and sequenced multiple independent products from polymerase chain reaction (PCR) amplified hT1R3 cDNAs derived from a human taste cDNA library. Based on the nucleotide sequence of the genomic DNA and cDNAs, the hydrophobicity profile and TMpred predictions of membrane

25   spanning regions (Figure 1C), hT1R3 is predicted to encode a protein of 843 amino acids with seven transmembrane helices and a large 558 amino acid long extracellular domain.

The corresponding mouse T1R3 (mT1R3) genomic sequence

30   was assembled from the Celera mouse genomic fragment database. Several reverse transcriptase (RT)-PCR-generated mouse T1R3 cDNAs derived from taste bud mRNA of different mouse strains were also cloned and sequenced. The coding portion of the mouse T1R3 gene from C57BL/6 spans 4 kb and

35   contains 6 exons; the encoded protein is 858 amino acids long. Polymorphic differences between taster and non-

37

taster strains of mice, and their potential functional significance, are described below (see Figures 5 and 6 and related text).

5   T1R3 is a member of the family 3 subtype of GPCRs, all of which contain large extracellular domains. Other family 3 subtype GPCRs include metabotropic glutamate receptors (mGluR), extracellular calcium sensing receptors (ECaSR), candidate pheromone receptors expressed in the vomeronasal organ (V2R), and two taste receptors, T1R1 and T1R2, of

10   unknown ligand specificity. T1R3 is most closely related to T1R1 and T1R2, sharing ~30% amino acid sequence identity with each of these orphan taste receptors (T1R1 and T1R2 are ~40% identical to each other). At the amino acid level hT1R3 is ~20% identical to mGluRs and ~23% identical to

15   ECaSRs. The large amino terminal domain (ATD) of family 3 GPCRs has been implicated in ligand binding and dimerization (19). Like other family 3 GPCRs, mT1R3 has an amino-terminal signal sequence, an extensive ATD of 573 amino acids, multiple predicted asparagine-linked

20   glycosylation sites (one of which is highly conserved), and several conserved cysteine residues. Nine of these cysteines are within a region that links the ATD to the portion of the receptor containing the transmembrane domains. The potential relevance of mT1R3's ATD in

25   phenotypic differences between taster and non-taster strains of mice is elaborated below (see Figures 5 and 6 and related text).

EXPRESSION OF T1R3 mRNA AND

PROTEIN IN TASTE TISSUE AND TASTE BUDS

30   To examine the general distribution of mouse T1R3 in taste and non-taste tissues, northern blot analysis was carried out with a panel of mouse mRNAs. The mouse T1R3 probe hybridized to a 7.2 kb mRNA present in taste tissue, but not expressed in control lingual tissue devoid of taste

35   buds (non-taste) or in any of the several other tissues

38

examined (Figure 2A). A somewhat larger (~7.8 kb) mRNA species was expressed at moderate levels in testis, and at very low levels in brain. A smaller (~6.7 kb) mRNA species was expressed at very low levels in thymus. The 7.2 kb

5   taste-expressed transcript is longer than the isolated cDNAs or Genscan predicted exons, suggesting that additional untranslated sequences may be present in the transcript.

As another measure of the pattern of expression of

10 T1R3 in various tissues the expressed sequence tags (est) database were examined for strong matches to T1R3 and other predicted genes in the Sac region (Figure 2B). While dvll, glycolipid transfer-like, cleavage and polyadenylation-like, and pseudouridine synthase-like genes each had

15 numerous highly significant matches to ests from several different tissues, T1R3 showed only a single strong match to an est from colon. This result, consistent with the northern, suggests that expression of T1R3 is highly restricted – such a pattern of under-representation in the

20 est database would fit with T1R3 being a taste receptor.

To determine the cellular pattern of T1R3 expression in taste tissue, in situ hybridization was performed: T1R3 was selectively expressed in taste receptor cells, but absent from the surrounding lingual epithelium, muscle or

25 connective tissue (Figure 3A). Sense probe controls showed no non-specific hybridization to lingual tissue (Figure 3A). The RNA hybridization signal for T1R3 was even stronger than that for α-gustducin (Figure 3A), suggesting that T1R3 mRNA is very highly expressed in taste receptor

30 cells. This is in contrast to results with T1R1 and T1R2 mRNAs, which are apparently expressed at lower levels than is α-gustducin (17). Furthermore, T1R3 is highly expressed in taste buds from fungiform, foliate and circumvallate papillae, whereas T1R1 and T1R2 mRNAs each show different

35 regionally variable patterns of expression (T1R1 is preferentially expressed in taste cells of the fungiform

39

papillae and geschmacksstreifen ('taste stripe'), to a
lesser extent in those of the foliate papillae, but rarely
in those of the circumvallate papillae; T1R2 is commonly
expressed in taste cells of the circumvallate and foliate
5   papillae, but rarely in those of the fungiform papillae or
geschmacksstreifen) (17).

     To determine if T1R3 mRNA is expressed in particular
subsets of taste receptor cells, expression profiling was
used (3). First, probes from the 3' regions of mouse
10   clones for T1R3, α-gustducin, Gγ13, PLCβ2 and G3PDH cDNAs
were hybridized to RT-PCR-amplified cDNAs from a single
circumvallate papilla vs. a similar-sized piece of non-
gustatory lingual epithelium. In this way it was
determined that mouse T1R3, like α-gustducin, Gγ13 and
15   PLCβ2, was expressed in taste bud-containing tissue, but
not in non-gustatory lingual epithelia (Fig. 3B left). The
pattern of expression of these genes in individual taste
cells was next profiled: the single cell RT-PCR products
were hybridized with the same set of probes used above. As
20   previously determined (3), all of the nineteen α-gustducin-
positive cells expressed Gβ3 and Gγ13; these nineteen cells
also all expressed PLCβ2 (Figure 3B right). Twelve of
these nineteen cells (63 %) also expressed T1R3. Only one
of the five cells that were α-gustducin/Gβ3/Gγ13/PLCβ2-
25   negative expressed T1R3. From this it was concluded that
expression of T1R3 and α-gustducin/Gβ3/Gγ13/PLCβ2, although
not fully coincident, overlaps to a great extent. This
contrasts with previous in situ hybridization results with
taste receptor cells of the foliate papillae in which ~15%
30   of α-gustducin-positive cells were positive for T1R1 or
T1R2 (17).

     Immunocytochemistry with an anti-hT1R3 antibody
demonstrated that about one fifth of taste receptor cells
in human circumvallate (Figure 4AC) and fungiform (Figure
35   4EH) papillae were positive for hTIR3. hTIR3
immunoreactivity was blocked by pre-incubation of the hTIR3

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antibody with the cognate peptide (Figure 4B).
Longitudinal sections of the hTIR3-postive taste cells
displayed an elongated bipolar morphology typical of so
called light cells (many of which are α-gustducin-
5   positive), with the immunoreactivity most prominent at or
near the taste pore (Figure 4ACEH). Labeling adjacent
sections with antibodies directed against hTIR3 and PLCß2
showed more cells positive for PLCß2 than for hT1R3 (Figure
4CD). Double labeling for hT1R3 and PLCß2 (Figure 4EFG),
10 or for hT1R3 and α-gustducin (Figure 4HIJ) showed many, but
not all, cells to be doubly positive (more cells were
positive for PLCß2 or α-gustducin than for hT1R3),
consistent with the results from expression profiling. In
sum, T1R3 mRNA and protein are selectively expressed in a
15 subset of "-gustducin /PLC$2- positive taste receptor cells
as would be expected for a taste receptor.

                    A SINGLE POLYMORPHIC DIFFERENCE
                    IN T1R3 MAY EXPLAIN THE SAC[d]
20   NON-TASTER PHENOTYPE

        C57BL/6 mice carrying the Sac[b] allele and other so-
called taster strains of mice display enhanced preferences
and larger chorda tympani nerve responses vs. DBA/2 mice
25 (sac[d]) and other non-taster strains for several compounds
that humans characterize as sweet (e.g. sucrose, saccharin,
acesulfame, dulcin and glycine) (10-12, 14, 15, 18). The
inferred amino acid sequence of T1R3 from taster and non-
taster strains of mice were examined looking for changes
30 that might explain these phenotypic differences (see Figure
5A). All four non-taster strains (DBA/2, 129/Svev, BALB/c
and C3H/HeJ) examined had identical nucleotide sequences
despite the fact that their most recent common ancestors
date back to the early 1900s or earlier (see Figure 5B).

                                41

All four taster strains (C57BL/6J, SWR ,FVB/N and ST/bj)
shared four nucleotide differences vs. the non-tasters:
$nt_{135}A \rightarrow G$, $nt_{163}A \rightarrow G$, $nt_{179}T \rightarrow C$ and $nt_{652}T \rightarrow C$ (the taster nt is
listed first). C57BL/6J also had a number of positions at

5   which it differed from all other strains (see Figure 5A),
however, many of these differences were either "silent"
alternate codon changes in protein coding regions or
substitutions within introns where they would be unlikely
to have any pronounced effect. The two coding changes

10   (described as single letter amino acid changes at specific
residues; the taster aa is listed first) were T55A and
I60T. The I60T change is a particularly intriguing
difference as it is predicted to introduce a novel N-linked
glycosylation site in the ATD of T1R3 (see below).

15   To consider the functional relevance of these two
amino acid differences in the T1R3 proteins from taster vs.
non-taster, the ATD of T1R3 was aligned with those of other
members of the type 3 subset of GPCRs (Figure 6) and the
ATD of T1R3 was modeled based on the recently solved

20   structure of the ATD of the related mGluR1 receptor (19)
(Figure 7). The ATD of T1R3 displays 28, 30, 24, and 20%
identity to those of T1R1, T1R2, CaSR and mGluR1,
respectively (Figure 6). 55 residues of ~570 in the ATD
were identical among all five receptors. Included among

25   these conserved residues is a predicted N-linked
glycosylation site at N85 of T1R3. Based on homology to
mGluR1, regions predicted to be involved in dimerization of
T1R3 are aa 55-60, 107-118, 152-160, and 178-181 (shown in

Fig. 6 within dashed boxes). The I60T taster to non-taster substitution is predicted to introduce a novel N-linked glycosylation site 27 amino acids upstream from the conserved N-linked glycosylation site present in all five

5 receptors. The new N-linked glycosylation site at N58 might interfere with normal glycosylation of the conserved site at N85, alter the structure of the ligand binding domain, interfere with potential dimerization of the receptor, or have some other effect on T1R3 function.

10 To determine if glycosylation at N58 of the non-taster variant of mT1R3 might be expected to alter the function of the protein we modeled its ATD on that of mGluR1 (19) (Figure 7). The regions of potential dimerization in T1R3 are very similar to those of mGluR1

15 and the amino acids in these regions form tight fitting contact surfaces that suggest that dimerization is indeed likely in T1R3. From the model of the three dimensional structure of the ATD of T1R3 we can see that the novel N-linked glycosylation site at N58 would have a profound

20 effect on T1R3's ability to dimerize (Figure 7C). The addition of even a short carbohydrate group at N58 (a tri-saccharide moiety has been added in the model in Figure 7C) would disrupt at least one of the contact surfaces required for stability of the dimer. Therefore, if T1R3, like

25 mGluR1, adopts a dimeric form (either homodimer or heterodimer), then the predicted N-linked glycosyl group at N58 would be expected to preclude T1R3 from forming self-homodimers or heterodimers with any other GPCRs co-

43

expressed with T1R3 using the same dimerization interface. Even if the novel predicted glycosylation site at N58 of non-taster T1R3 is not utilized, theT55A and I60T substitutions at the predicted surface of dimerization may

5   themselves affect the ability of T1R3 to form dimers.

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25   The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in

46

the art from the foregoing description and accompanying

figures.   Such modifications are intended to fall within

the scope of the appended claims.   Various references are

cited herein, the disclosures of which are incorporated by

5   reference in their entireties.

WE CLAIM:

1.   An isolated nucleic acid molecule
comprising a nucleotide sequence that encodes the amino
acid sequence shown in Figure 1B.

5   2.   The isolated nucleic acid molecule of
claim 1 comprising the DNA sequence of Figure 1B.

3.   The isolated nucleic acid molecule of
claim 2 comprising a nucleotide sequence that encodes the
amino acid sequence shown in Figure 1B.

10   4.   An isolated nucleic acid molecule
comprising a nucleotide sequence that hybridizes to the
nucleotide sequence of Claim 1 or 2 under stringent
conditions and encodes a functionally equivalent gene
product.

15   5.   An isolated nucleic acid molecule
comprising a nucleotide sequence that hybridizes to the
nucleic acid of claim 1 or 2 under moderately stringent
conditions and encodes a functionally equivalent T1R3
gene product.

20   6.   An isolated nucleic acid molecule that is
a T1R3 antisense molecule.

7.   An isolated polypeptide comprising the
amino acid sequence of Figure 1B.

8.   An isolated polypeptide comprising the
25 amino acid sequence encoded by a nucleotide sequence that
hybridizes to the nucleotide sequence of Claim 1 or 2
under stringent conditions and encodes a functionally

48

equivalent gene product.

9.   An isolated polypeptide comprising the amino acid sequence encoded by a nucleotide sequence that hybridizes to the nucleotide sequence of Claim 1 or 2

5   under moderately stringent conditions and encodes a functionally equivalent gene product.

10.   A purified fragment of a T1R3 protein comprising a domain of the T1R3 protein selected from the group consisting of the amino terminal domain,

10   transmembrane domain and cytoplasmic domain.

11.   A chimeric protein comprising a fragment of a T1R3 protein consisting of at least 6 amino acids fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not a T1R3

15   protein.

13.   An antibody which is capable of binding a T1R3 protein.

A recombinant cell containing the nucleic acid of claim 4 or 5.

20   14.   A method of producing a T1R3 protein comprising growing a recombinant cell containing the nucleic acid of claim 4 or 5 such that the encoded T1R3 protein is expressed by the cell, and recovering the expressed T1R3 protein.

25   15.   A method for identifying a compound that induces the perception of a sweet taste comprising:

(i)   contacting a cell expressing the T1R3 channel protein with

49

a test compound and measuring the

level of T1R3

activation;

(ii) in a separate experiment, contacting

5   a cell expressing the

T1R3 receptor protein with a vehicle

control and measuring

the level of T1R3 activation where

the conditions are

10   essentially the same as in part (i);

and

(iii)   comparing the level of

activation of T1R3 measured in

part (i) with the level of activation

15   of T1R3 in part (ii),

wherein an increased level of activated T1R3 in the

presence of the test compound indicates that the test

compound is a T1R3 inducer.

16. A method for identifying a compound that

20   inhibits the perception of a sweet taste and/or promotes

the perception of a sweet taste comprising:

(i) contacting a cell expressing the T1R3

receptor protein with a test

compound in the presence of a sweet

25   tastant and measuring the

level of T1R3 activation;

(ii) in a separate experiment, contacting a

cell expressing the T1R3

receptor protein with a sweet tastant and measuring the level of

T1R3 activation, where the conditions are essentially the same as

5   in part (i); and

(iii)   comparing the level of activation of T1R3 measured in part (i) with

the level of activation of T1R3 in part (ii),

10   wherein a decrease level of activation of T1R3 in the presence of the test compound indicates that the test compound is a T1R3 inhibitor.

17.   A method for identifying an inhibitor of sweet taste _in vivo_ comprising:

15   (i)   offering a test animal the choice of consuming either (a) a

composition comprising a sweet tastant or (b) the

composition comprising the sweet

20   tastant as well as a test

inhibitor; and

(ii) comparing the amount of consumption of the composition

according to (a) or (b),

25   wherein greater consumption of the composition according to (a) has a positive correlation with an ability of the test inhibitor to inhibit the perception of sweet taste associated with the tastant.

51

18. A method for identifying an activator of sweet taste _in vivo_ comprising:

      (i) offering a test animal the choice of consuming either (a) a

5   control composition or (b) the composition comprising a

             test activator; and

      (ii) comparing the amount of consumption of the composition

10   according to (a) or (b),

wherein greater consumption of the composition according to (b) has a positive correlation with an ability of the test activator to activate the perception of sweet taste.

19. A method of inhibiting a sweet taste

15   resulting from contacting a taste tissue of a subject with a sweet tastant, comprising administering to the subject an effective amount of a T1R3 inhibitor.

20. A method of producing the perception of a sweet taste by a subject, comprising administering, to

20   the subject, a composition comprising a compound that acts as an activator of T1R3.

21. A method of producing the perception of a sweet taste by a subject, comprising administering, to the subject, a composition comprising a compound that

25   acts as a sweetness activator.

52
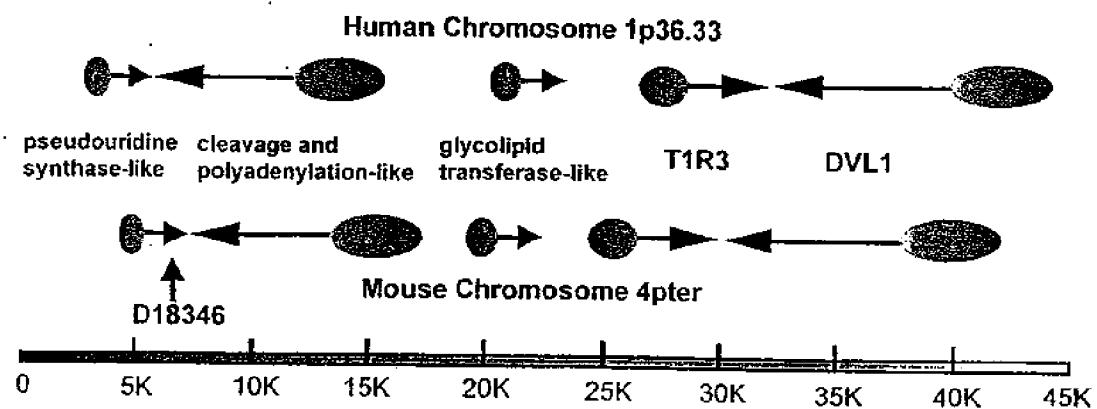
FIG. 1A

FIG. 1B
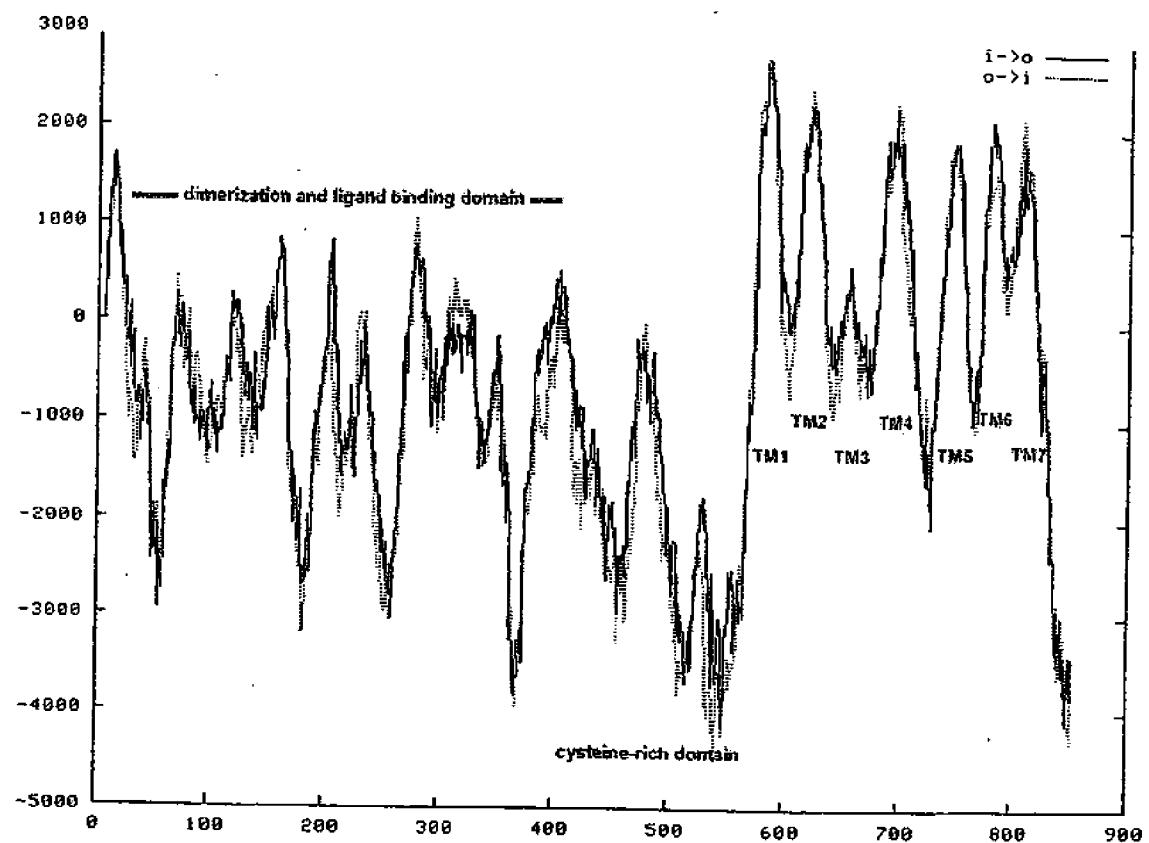
FIG. 1C
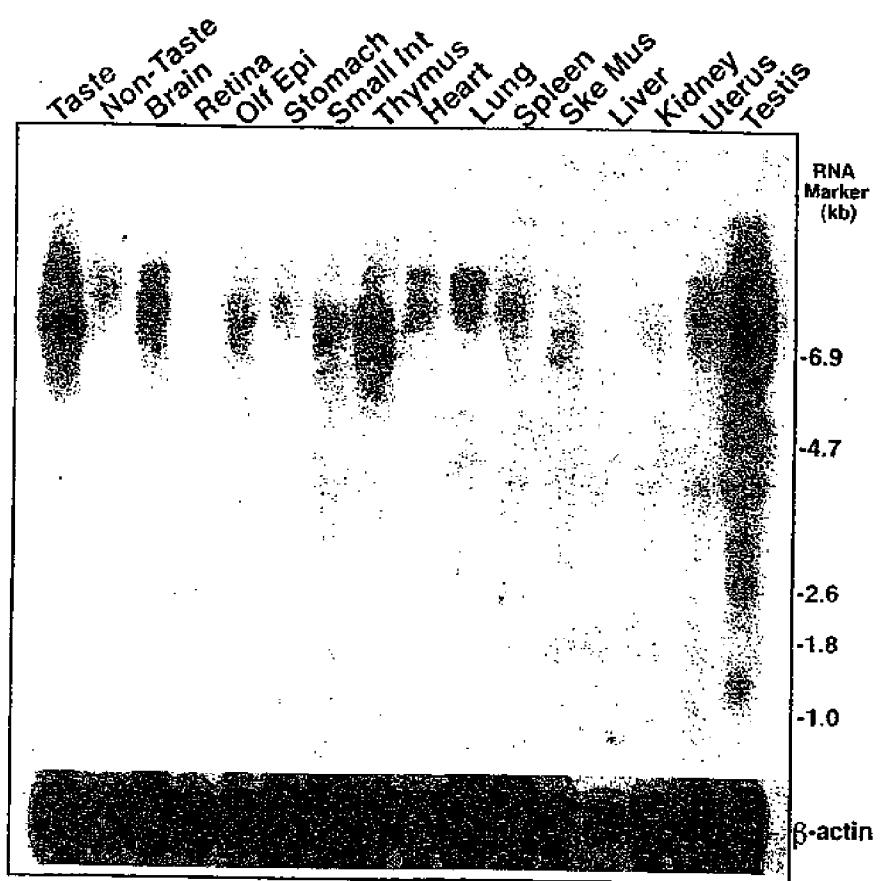
FIG. 2A
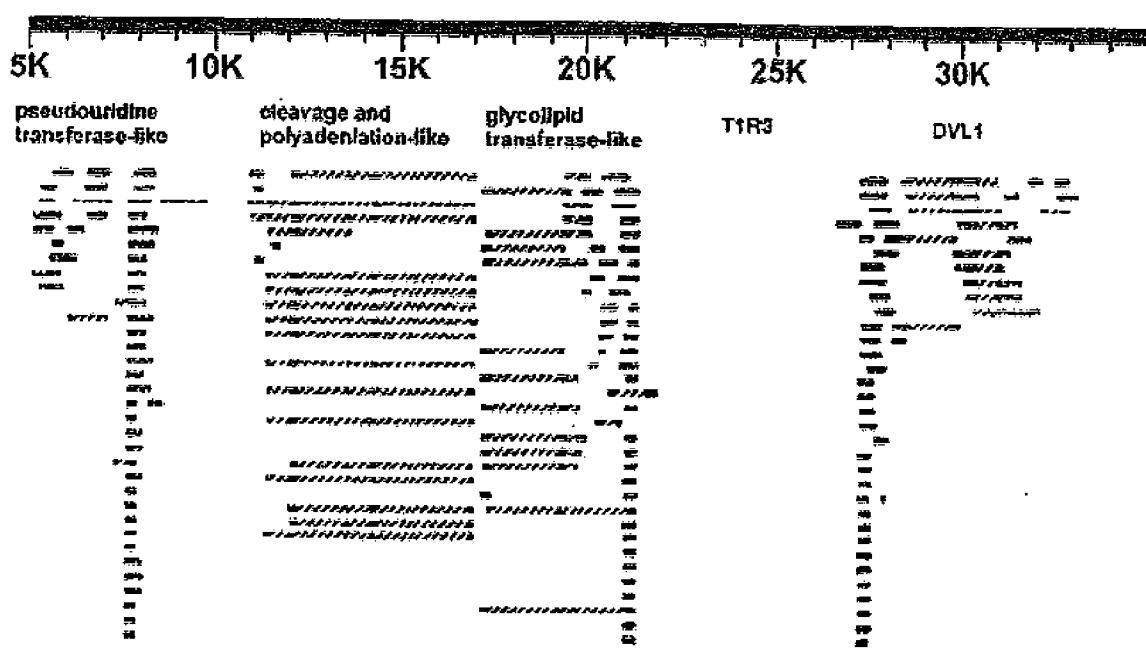
FIG. 2B
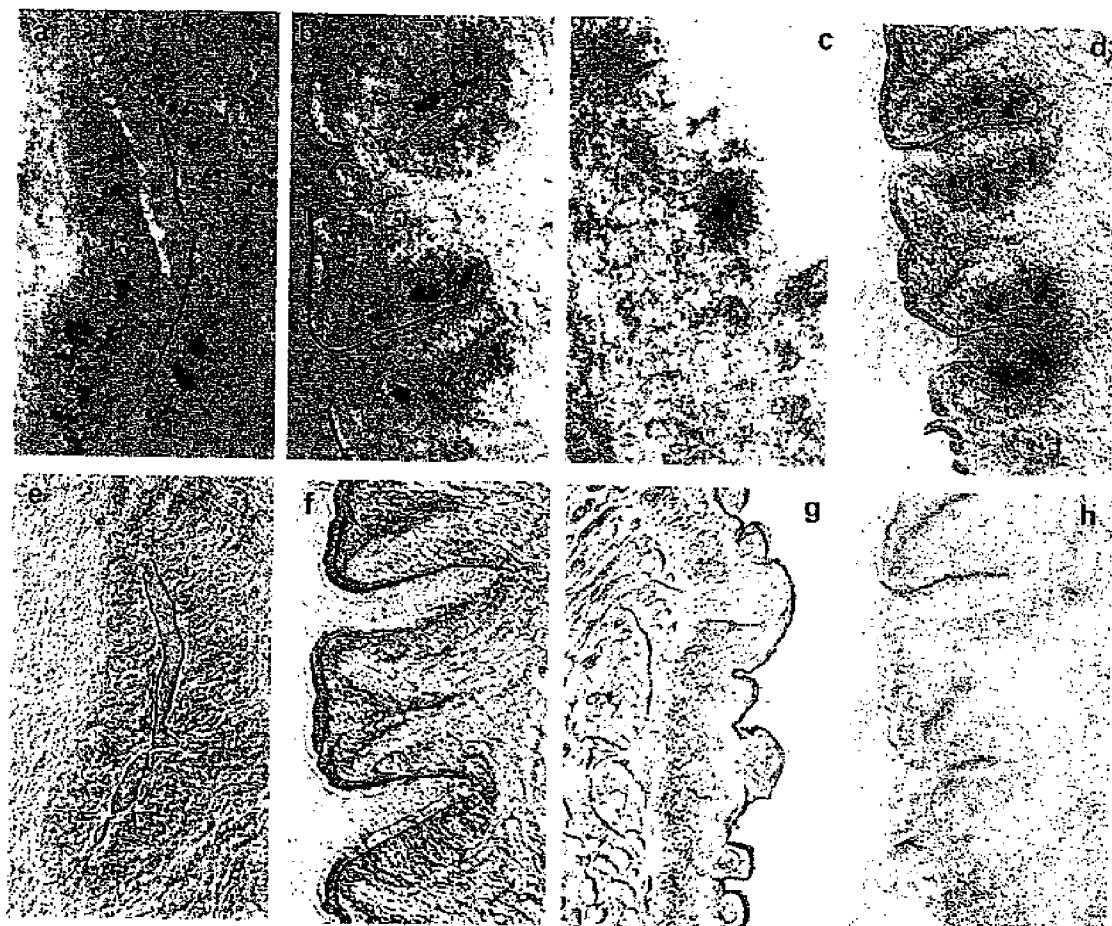
FIG. 3A
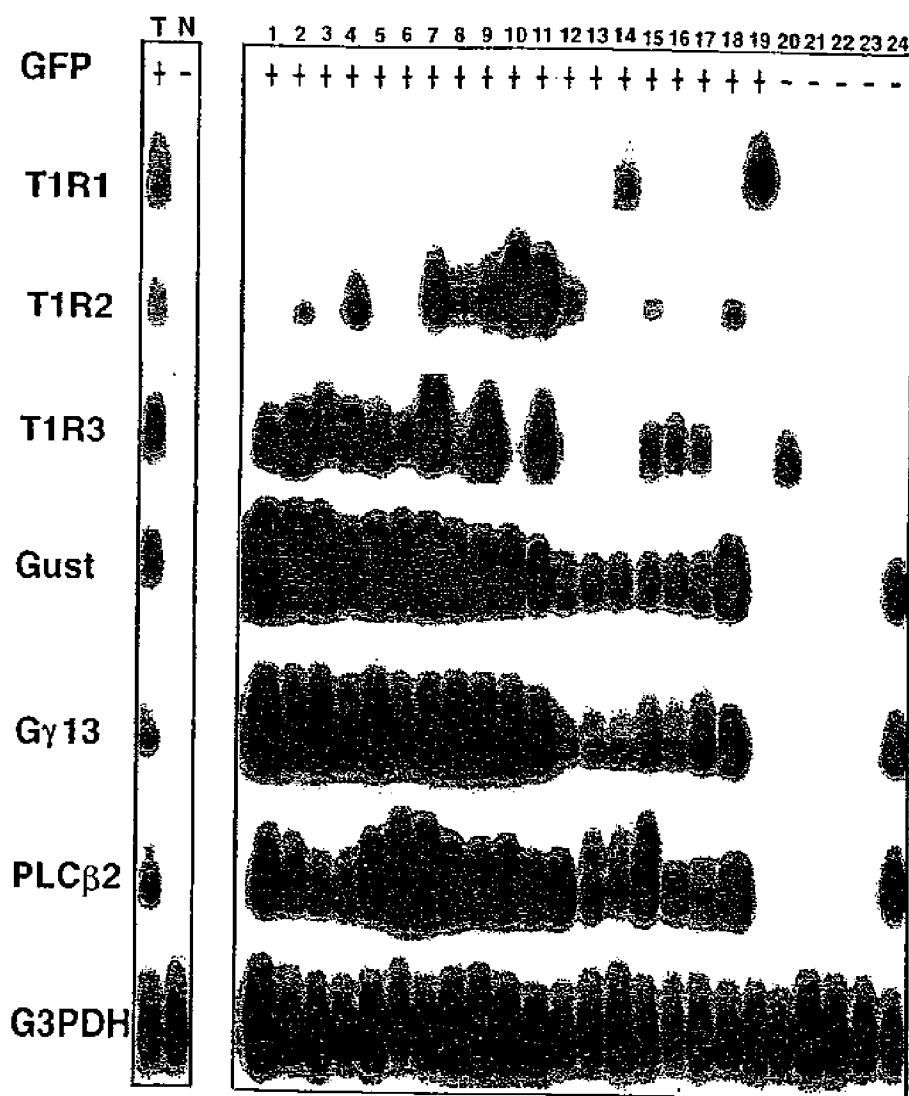
FIG. 3B
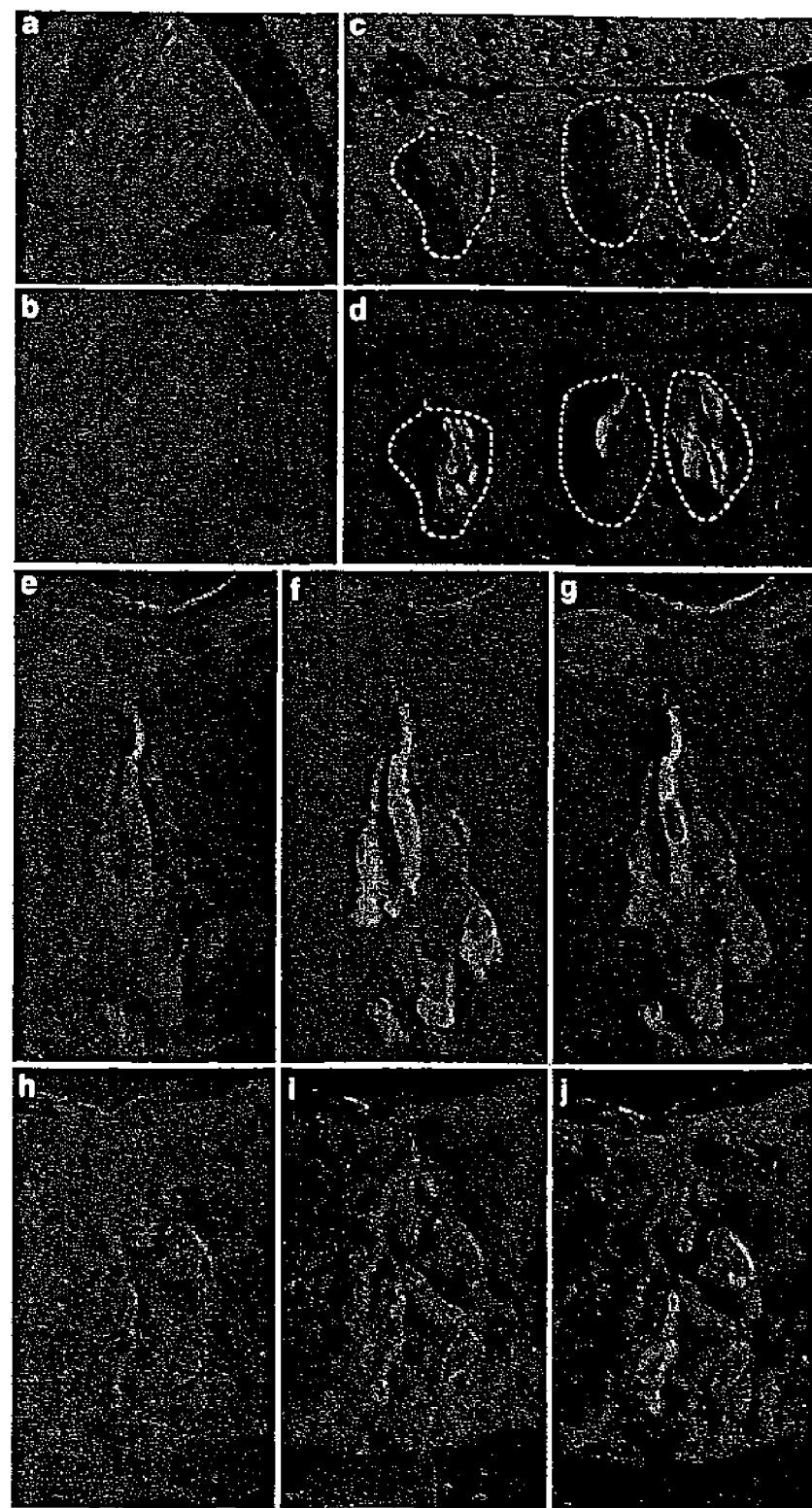
FIG. 4

Nucleotide position	135	163	179	182	186	264	270	312	652	692	965	969	1300	2647	2689
C57BL/6J	A	A	T	C	C	-	A	T	T	T	A	C	G	T	T
FVB/N, SWR, ST/bJ	A	A	T	T	T	G	G	C	T	C	G	T	A	C	C
Non-taster strains	G	G	C	T	T	G	G	C	C	C	G	C	A	T	C
Coding change	s	T55A	I60T	P61L	s	i	i	s	i	s	s	C261R	R371Q	S692L	I706T

FIG. 5A

Danish outbred
white mice ————————————————————————————————— ST/bJ   (1940)

Swiss mice ┌─── Lynch (1926) ——————————————————————— SWR   (1926)
           └─── NIH (1935) ————————————————————————— FVB/N   (1970s)

Lathrop   ┌─── Little (1921) ——————————————————————— C57BL/6 (1921)
Granby   │
1903-1915 ─┤   English fanciers ─── Dunn (1928) ——————— 129   (1928)
           │
           └─── Castle
                      └─── Little (1909) ———————————————— DBA/2   (1909)
                                  └─── Strong (1920) ——————— C3H/He (1920)
Carnegie Institution

Ohio dealer ————————————————————————————————— BALB/c (1913)
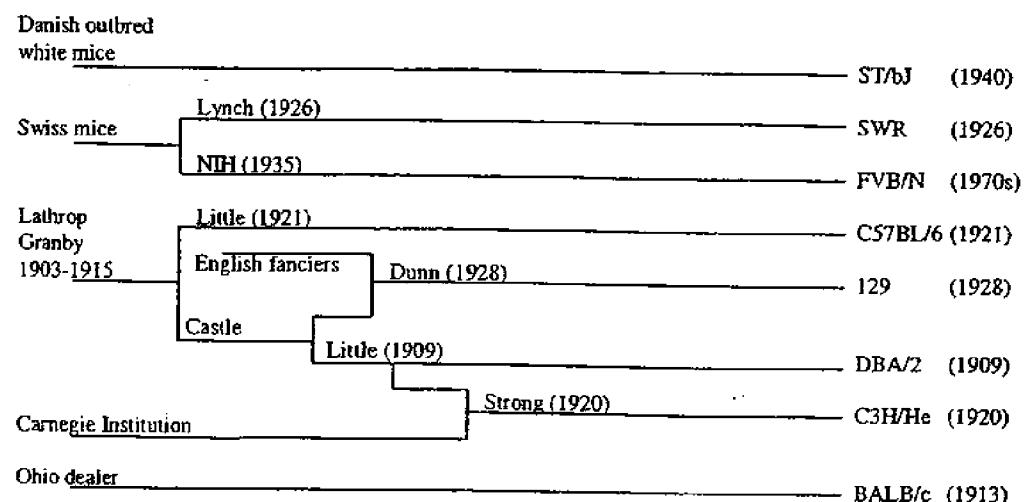
FIG. 5B

```
                    predicted leader sequence
mT1R3   -----MPALAIMGLSLAAFLELGMGASLCLSQQFKAQ   YILGG   PLGSTEEATLN-QRTQPNSIP NRFSP---L LFLAM   KMAV-80
rT1R1   ---MLFWAAHLLLSLQLVYCWAFSCQRTESSPGFSLP   FLLAG   SLHGDCLQV----IRHRPLVTS DRPDSFNGH YHLPQ   RFTV
rT1R2   ----MGPQARTLCLLSLLLHVLPKPGKLVENSDFHLA   YLLGG   TLHANVKSIS--HLSYLQVPK NEFTMKV-L YNLMQ   RFAV
mECaSR  -------MAWFGYCLALLALTWHSSAYGPDQRAQKK   IILGG   PIHFGVSAKDQDIKSRPESVE IRYNFR--- FRWLQ   IFAI
mGluR1  ..FFPMIFLEMSILPRMPDRKVLLAGASSQRSVARMD   VIIGA   SVHHQPP------AEKVPERK GEIREQ--Y IQRVE   FHTL

mT1R3   EE  NGSA   GLR   YDLF T SEPVVTMKSSLMFLAKVGSQ-----SIAAYCNY-------TQYQPRVLAVI PHSSELALITGKFFl 157
rT1R1   EE  NSSA   NIT   YELY V SESANVYATLRVLALQGPR------HIEIQKDL--------RNHSSKVVAFI PDNTDHAVITAALLl
rT1R2   EE  NCSS   GVL   YEMV V YLSNNIHPGLYFLAQDDD-------LLPILKDY--------SQYMPHVVAVI PDNSESAIIVSNILl
mECaSR  EE  SSPA   NMT   YRIF T NTVSKALEATLSFVAQNKIDS---LNLDEFCNC------SEHIPSTIAVV ATGSGVSTAVANLLl
mGluR1  DK  ADPV   NIT   SEIR S WHSSVALEQSIEFIRDSLISIRDEKDGLNRCLPDGQTLPPGRTKKPIAGVI PGSSSVAIQVQNLLl

mT1R3   lSF lLM QVS SASMDR SDRETFPSFF TV SDRVQLQAVVTLLQNFS NWVAALGSDDD   REGLSIFSS-LANARGI IAHEGLVPQ-246
rT1R1   lGP lLM LVS EASSVV SAKRKFPSFL TV SDRHQVEVMVQLLQSFG VWISLIGSYGD   QLGVVQALEE-LAVPRGI VAFKDIVPF
rT1R2   lSH lLI QIT SAISDK RDKRHFHEML TV SATHHIEAMVQLMVHFQ NWIVVLVSDDD   RENSHLLSQRLTKTSDI IAFQEVLPI
mECaSR  lGL lYI QVS ASSSRL SNKNQFKSFL TI NDEHQATAMADIIEYFR NWVGTIAADDD   RPGIEKFREEAEER-DI IDFSELISQ
mGluR1  lOL lPI QIA SATSID SDKTLYKYFL VV SDTLQARAMLDIVKRYN TYVSAVHTEGN   ESGMDAFKELAAQE-GL IAHSDKIYS

mT1R3   HDTS----GQQLGKVLDVLRQVNQSKVQ VVL ASARAVYSLFSYSIHHGLSPK-VWVA ES LTSDLVMTLPNIARV TVLGFLQRGA-331
rT1R1   S------ARVGDPR-MQSMMQHLAQARTT VVV SNRHLARVFFRSVVLANLTGK-VWVA ED AISTYITSVTGIQGI TVLGVAVQQR
rT1R2   PESSQVMRSEEQRQLDNILDKLRRTSAR VVV SPELSLYSFPHEVLRWNFTG-FVWIA ES AIDPVLHNLTELRHT TFLGVTIQRV
mECaSR  Y--------SDEREIQQVVEVIQNSTAK IVV SSGPDLEPLIKEIVRRNITGR-IWLA EA ASSSLIAMPEYFHVV GTIGFGLKAG
mGluR1  N------AGEKSPDRLLRKLRERLPKAR VVC CEGMTVRGLLSAMRRLGVVGEFSLIG DG ADRDEVIEGYEVEAN GITIKLQSPE

mT1R3   LLPEFSHYVE-----------THLALAADPAF ASLNAELDLEEHV---------------MGQRCPR DDIMLQNLSSGLLQNLSAG-393
rT1R1   QVPGLKEFEE----------SYVRAVTAAPSA PEGSWSTC-----------------------NQL RECHTFTTRNMPTLGAFSM
rT1R2   SIPGFSQFRV----RRDKPGYPVPNTTNLRTT ----------------------------NQD DACLNTTKSFNNILILSGE
mECaSR  QIPGFREFLQKVHPRKSVHNGFAKEFWEETFN HLQDGAKGPLPVDTFVRSHEEGGNRLLNSSTAFRPL TGDENINSVETPYMDYEHL
mGluR1  VRS-FDDYFLKLRLDTNTRNPWFPEFWQHRFQ RLPGHLL------------------ENPNFKKV TGNESL--EENYVQDSK--

mT1R3   QLHHQIFATYA V SV QA HNTLQ-- N------VSH HVSEHVLPWQL ENM-YNMS HARDLTLQ-   AE NVDMEYDLKMWVWQ-471
rT1R1   SAA---YRVYE V AV HG HQLLG-- T------SEI SRG-PVYPWQL QQI-YKVN LLHENTVA-   DN DTLGYYDIIAWDWN
rT1R2   RVV---YSVYS V AV HA HRLLG-- N------RVR TKQ-KVYPWQL REI-WHVN TLLGNRLF-   QQ DMPMLLDIIQWQWD
mECaSR  RIS---YNVYL V SI HA QDIYT-- LPGRGLFTNGS ADIKKVEAWQV KHLR-HLN TNNMGEQVT   EC DLVGNYSIINWHLS
mGluR1  ----MGF-VIN I AM HG QNMHHAL PG-----HVGL DAMKPIDGRKL DFLI-KSS VGVSGEEVW   EK DAPGRYDIMNLQYT

mT1R3   SPTPVLHTVGTFNGT-------- QLQQSKMYWPG--NQ PV Q SRQ KD QVRRVKGFH-S   YD VD KAGSYR-KHPDDFT TP -548
rT1R1   GPEWTFEIIGSASLSPVH----- DINKTKIQWHGKNNQ PV V TTD LA HHRVVVGSH-H   FE VP EAGTFL-NMSELHI QP
rT1R2   LSQNPFQSIASYSPTSKR----- TYIN-NVSWYTPNNT PV M SKS QP QMKKSVGLH-P   FE LD MPGTYLNRSADEFN LS
mECaSR  PEDGSIVPKEVGYYNVYAKKGER FINEGKILWSGFSRE PF N SRD QA TRKGIIEGEPT   FE AE PDG-EYSGETDASA DK
mGluR1  EAN-RYDYVHVGTWHECV----- NIDDYKIQMNK-SGM -R V SEP LK QIKVIRKGEVS   WI TA KENEFVQ---DEFT RA
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FIG. 6
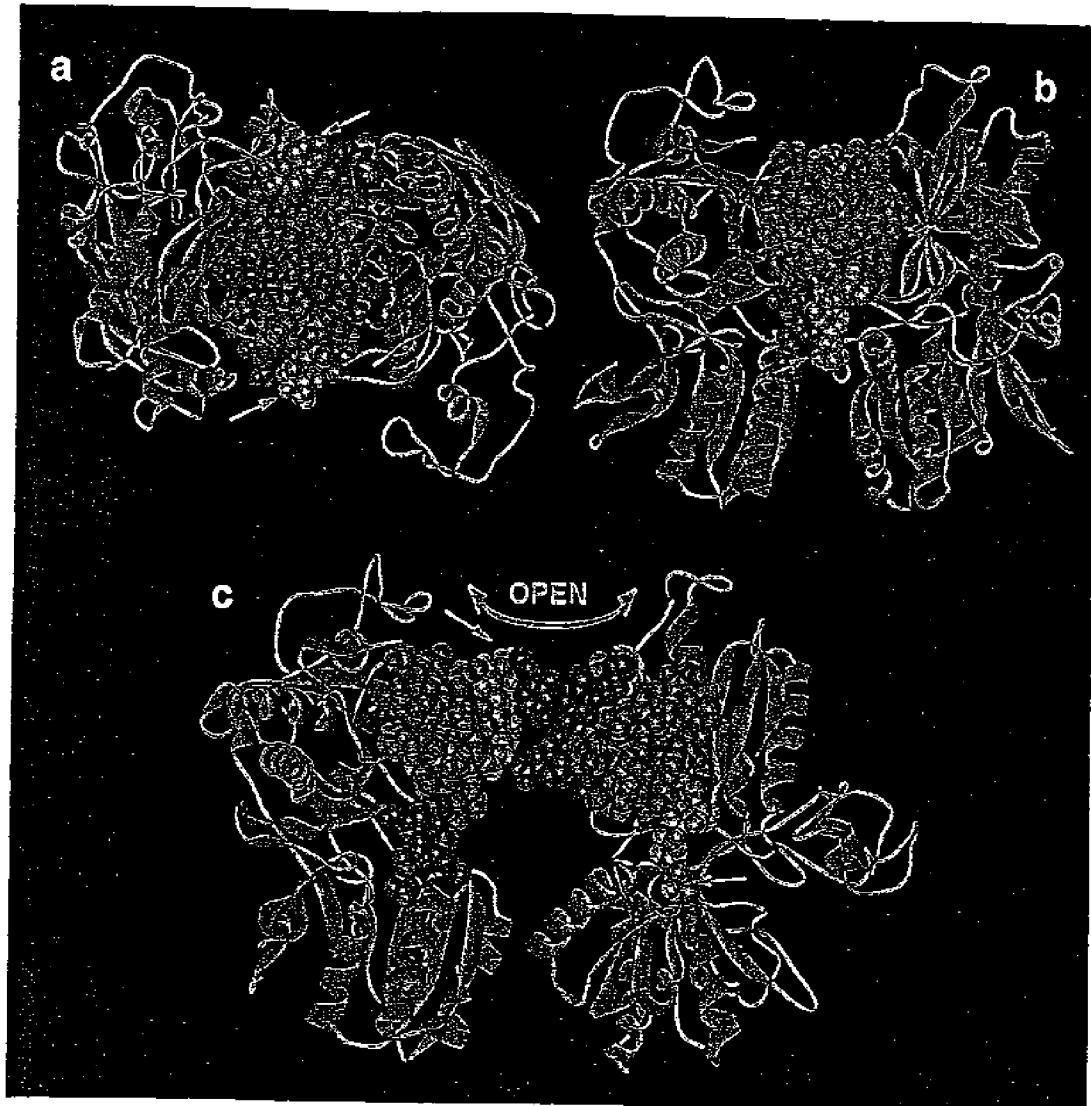
FIG. 7

SEQUENCE LISTING

<110>   Margolskee et al.

<120>   T1R3 A NOVEL TASTE RECEPTOR

<130>   1279-001

<140>   60/285,209

<141>   2001-04-20

<150>   Not applicable.

<151>   Not applicable.

<160>   7

<210>   1

<211>   343

<212>   DNA

<213>   Homo sapiens

<220>   Feature:

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ctgccgtgcc   tgttggaagt   tgcctctgcc   atg   ctg   ggc   cct   gct   gtc   ctg   171
                                        Met   Leu   Gly   Pro   Ala   Val   Leu
                                        1   5

ggc   ctc   agc   ctc   tgg   gct   ctc   ctg   cac   cct   ggg   acg   ggg   210
Gly   Leu   Ser   Leu   Trp   Ala   Leu   Leu   His   Pro   Gly   Thr   Gly
             10   15   20

1

gcc	cca	ttg	tgc	ctg	tca	cag	caa	ctt	agg	atg	aag	ggg	249
Ala	Pro	Leu	Cys	Leu	Ser	Gln	Gln	Leu	Arg	Met	Lys	Gly	
				25					30				

gac	tac	gtg	ctg	ggg	ggg	ctg	ttc	ccc	ctg	ggc	gag	gcc	288
Asp	Tyr	Val	Leu	Gly	Gly	Leu	Phe	Pro	Leu	Gly	Glu	Ala	
	35					40					45		

gag	gag	gct	ggc	ctc	cgc	agc	cgg	aca	cgg	ccc	agc	agc	327
Glu	Glu	Ala	Gly	Leu	Arg	Ser	Arg	Thr	Arg	Pro	Ser	Ser	
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Pro	Val	Cys	Thr	Arg		
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		Phe	Ser	Ser	Asn	Gly	Leu	Leu	Trp	Ala	Leu	Ala	Met	
		1				5					10			

aaa	atg	gcc	gtg	gag	gag	atc	aac	aac	aag	tcg	gat	ctg	ctg	424
Lys	Met	Ala	Val	Glu	Glu	Ile	Asn	Asn	Lys	Ser	Asp	Leu	Leu	
		15					20					25		

ccc	ggg	ctg	cgc	ctg	ggc	tac	gac	ctc	ttt	gat	acg	tgc	tcg	466
Pro	Gly	Leu	Arg	Leu	Gly	Tyr	Asp	Leu	Phe	Asp	Thr	Cys	Ser	
			30					35					40	

2

gag	cct	gtg	gtg	gcc	atg	aag	ccc	agc	ctc	atg	ttc	ctg	gcc	508
Glu	Pro	Val	Val	Ala	Met	Lys	Pro	Ser	Leu	Met	Phe	Leu	Ala	
				45					50					

aag	gca	ggc	agc	cgc	gac	atc	gcc	gcc	tac	tgc	aac	tac	acg	550
Lys	Ala	Gly	Ser	Arg	Asp	Ile	Ala	Ala	Tyr	Cys	Asn	Tyr	Thr	
55					60					65				

cag	tac	cag	ccc	cgt	gtg	ctg	gct	gtc	atc	ggg	ccc	cac	tcg	592
Gln	Try	Gln	Pro	Arg	Val	Leu	Ala	Val	Ile	Gly	Pro	His	Ser	
	70					75					80			

tca	gag	ctc	gcc	atg	gtc	acc	ggc	aag	ttc	ttc	agc	ttc	ttc	634
Ser	Glu	Leu	Ala	Met	Val	Thr	Gly	Lys	Phe	Phe	Ser	Phe	Phe	
		85					90					95		

ctc	atg	ccc	cag	gt										648
Leu	Met	Pro	Gln											
			100											

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	Val	Ser	Tyr	Gly	Ala	Ser	Met	Glu	Leu	Leu	Ser	Ala	Arg	
	1				5					10				

gag	acc	ttc	ccc	tcc	ttc	ttc	cgc	acc	gtg	ccc	agc	gac	cgt	731
Glu	Thr	Phe	Pro	Ser	Phe	Phe	Arg	Thr	Val	Pro	Ser	Asp	Arg	
	15					20					25			

gtg	cag	ctg	acg	gcc	gcc	gcg	gag	ctg	ctg	cag	gag	ttc	ggc	773
Val	Gln	Leu	Thr	Ala	Ala	Ala	Glu	Leu	Leu	Gln	Glu	Phe	Gly	
		30					35					40		

3

tgg	aac	tgg	gtg	gcc	gcc	ctg	ggc	agc	gac	gac	gag	tac	ggc	815
Trp	Asn	Trp	Val	Ala	Ala	Leu	Gly	Ser	Asp	Asp	Glu	Tyr	Gly	
			45					50					55	

cgg	cag	ggc	ctg	agc	atc	ttc	tcg	gcc	ctg	gcc	gcg	gca	cgc	857
Arg	Gln	Gly	Leu	Ser	Ile	Phe	Ser	Ala	Leu	Ala	Ala	Ala	Arg	
				60					65					

ggc	atc	tgc	atc	gcg	cac	gag	ggc	ctg	gtg	ccg	ctg	ccc	cgt	899
Gly	Ile	Cys	Ile	Ala	His	Glu	Gly	Leu	Val	Pro	Leu	Pro	Arg	
70					75					80				

gcc	gat	gac	tcg	cgg	ctg	ggg	aag	gtg	cag	gac	gtc	ctg	cac	941
Ala	Asp	Asp	Ser	Arg	Leu	Gly	Lys	Val	Gln	Asp	Val	Leu	His	
	85					90					95			

cag	gtg	aac	cag	agc	agc	gtg	cag	gtg	gtg	ctg	ctg	ttc	gcc	983
Gln	Val	Asn	Gln	Ser	Ser	Val	Gln	Val	Val	Leu	Leu	Phe	Ala	
		100					105					110		

tcc	gtg	cac	gcc	gcc	cac	gcc	ctc	ttc	aac	tac	agc	atc	agc	1025
Ser	Val	His	Ala	Ala	His	Ala	Leu	Phe	Asn	Try	Ser	Ile	Ser	
			115					120					125	

agc	agg	ctc	tcg	ccc	aag	gtg	tgg	gtg	gcc	agc	gag	gcc	tgg	1067
Ser	Arg	Leu	Ser	Pro	Lys	Val	Trp	Val	Ala	Ser	Glu	Ala	Trp	
				130					135					

ctg	acc	tct	gac	ctg	gtc	atg	ggg	ctg	ccc	ggc	atg	gcc	cag	1109
Leu	Thr	Ser	Asp	Leu	Val	Met	Gly	Leu	Pro	Gly	Met	Ala	Gln	
140					145					150				

atg	ggc	acg	gtg	ctt	ggc	ttc	ctc	cag	agg	ggt	gcc	cag	ctg	1151
Met	Gly	Thr	Val	Leu	Gly	Phe	Leu	Gln	Arg	Gly	Ala	Gln	Leu	
	155					160					165			

cac	gag	ttc	ccc	cag	tac	gtg	aag	acg	cac	ctg	gcc	ctg	gcc	1193
His	Glu	Phe	Pro	Gln	Tyr	Val	Lys	Thr	His	Leu	Ala	Leu	Ala	
		170					175					180		

acc	gac	ccg	gcc	ttc	tgc	tct	gcc	ctg	ggc	gag	agg	gag	cag	1235
Thr	Asp	Pro	Ala	Phe	Cys	Ser	Ala	Leu	Gly	Glu	Arg	Glu	Gln	
			185					190					195	

4

ggt	ctg	gag	gag	gac	gtg	gtg	ggc	cag	cgc	tgc	ccg	cag	tgt	1277
Gly	Leu	Glu	Glu	Asp	Val	Val	Gly	Gln	Arg	Cys	Pro	Gln	cys	
				200					205					

gac	tgc	atc	acg	ctg	cag	aac	gtg	agc	gca	ggg	cta	aat	cac	1319
Asp	Cys	Ile	Thr	Leu	Gln	Asn	Val	Ser	Ala	Gly	Leu	Asn	His	
210					215					220				

cac	cag	acg	ttc	tct	gtc	tac	gca	gct	gtg	tat	agc	gtg	gcc	1361
His	Gln	Thr	Phe	Ser	Val	Tyr	Ala	Ala	Val	Tyr	Ser	Val	Ala	
	225					230					235			

cag	gcc	ctg	cac	aac	act	ctt	cag	tgc	aac	gcc	tca	ggc	tgc	1403
Gln	Ala	Leu	His	Asn	Thr	Leu	Gln	Cys	Asn	Ala	Ser	Gly	Cys	
		240					245					250		

ccc	gcg	cag	gac	ccc	gtg	aag	ccc	tgg	cag		gt			1435
Pro	Ala	Gln	Asp	Pro	Val	Lys	Pro	Trp	Gln					
			255					260						

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<212> Type: DNA

<213> Homo sapiens

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	1				5					10				

ggg	ctg	ccg	ctg	cgg	ttc	gac	agc	agc	gga	aac	gtg	gac	atg	1518
Gly	Leu	Pro	Leu	Arg	Phe	Asp	Ser	Ser	Gly	Asn	Val	Asp	Met	
	15					20					25			

gag	tac	gac	ctg	aag	ctg	tgg	gtg	tgg	cag	ggc	tca	gtg	ccc	1560
Glu	Tyr	Asp	Leu	Lys	Leu	Trp	Val	Trp	Gln	Gly	Ser	Val	Pro	
		30					35					40		

agg	ctc	cac	gac	gtg	ggc	agg	ttc	aac	ggc	agc	ctc	agg	aca	1602
Arg	Leu	His	Asp	Val	Gly	Arg	Phe	Asn	Gly	Ser	Leu	Arg	Thr	
			45					50					55	

5

gag	cgc	ctg	aag	atc	cgc	tgg	cac	acg	tct	gac	aac	cag	gt	1643
Glu	Arg	Leu	Lys	Ile	Arg	Trp	His	Thr	Ser	Asp	Asn	Gln		
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	Lys	Pro	Val	Ser	Arg	Cys	Ser	Arg	Gln	Cys	Gln	Glu	Gly	
	1				5					10				

cag	gtg	cgc	cgg	gtc	aag	ggg	ttc	cac	tcc	tgc	tgc	tac	gac	1726
Gln	Val	Arg	Arg	Val	Lys	Gly	Phe	His	Ser	Cys	Cys	Tyr	Asp	
	15					20					25			

tgt	gtg	gac	tgc	gag	gcg	ggc	agc	tac	cgg	caa	aac	cca	g	1766
Cys	Val	Asp	Cys	Glu	Ala	Gly	Ser	Tyr	Arg	Gln	Asn	Pro		
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gt														1768

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	Asp	Asp	Ile	Ala	Cys	Thr	Phe	Cys	Gly	Gln	Asp	Glu	Trp	Ser	
	1				5					10					

ccg	gag	cga	agc	aca	cgc	tgc	ttc	cgc	cgc	agg	tct	cgg	ttc	1853
Pro	Glu	Arg	Ser	Thr	Arg	Cys	Phe	Arg	Arg	Arg	Ser	Arg	Phe	
15					20					25				

ctg	gca	tgg	ggc	gag	ccg	gct	gtg	ctg	ctg	ctg	ctc	ctg	ctg	1895
Leu	Ala	Trp	Gly	Glu	Pro	Ala	Val	Leu	Leu	Leu	Leu	Leu	Leu	
	30					35						40		

ctg	agc	ctg	gcg	ctg	ggc	ctt	gtg	ctg	gct	gct	ttg	ggg	ctg	1937
Leu	Ser	Leu	Ala	Leu	Gly	Leu	Val	Leu	Ala	Ala	Leu	Gly	Leu	
		45					50					55		

ttc	gtt	cac	cat	cgg	gac	agc	cca	ctg	gtt	cag	gcc	tcg	ggg	1979
Phe	Val	His	His	Arg	Asp	Ser	Pro	Leu	Val	Gln	Ala	Ser	Gly	
			60					65					70	

ggg	ccc	ctg	gcc	tgc	ttt	ggc	ctg	gtg	tgc	ctg	ggc	ctg	gtc	2021
Gly	Pro	Leu	Ala	Cys	Phe	Gly	Leu	Val	Cys	Leu	Gly	Leu	Val	
				75					80					

tgc	ctc	agc	gtc	ctc	ctg	ttc	cct	ggc	cag	ccc	agc	cct	gcc	2063
Cys	Leu	Ser	Val	Leu	Leu	Phe	Pro	Gly	Gln	Pro	Ser	Pro	Ala	
85					90					95				

cga	tgc	ctg	gcc	cag	cag	ccc	ttg	tcc	cac	ctc	ccg	ctc	acg	2105
Arg	Cys	Leu	Ala	Gln	Gln	Pro	Leu	Ser	His	Leu	Pro	Leu	Thr	
	100					105					110			

ggc	tgc	ctg	agc	aca	ctc	ttc	ctg	cag	gcg	gcc	gag	atc	ttc	2147
Gly	Cys	Leu	Ser	Thr	Leu	Phe	Leu	Gln	Ala	Ala	Glu	Ile	Phe	
		115					120					125		

gtg	gag	tca	gaa	ctg	cct	ctg	agc	tgg	gca	gac	cgg	ctg	agt	2189
Val	Glu	Ser	Glu	Leu	Pro	Leu	Ser	Trp	Ala	Asp	Arg	Leu	Ser	
			130					135						140

ggc	tgc	ctg	cgg	ggg	ccc	tgg	gcc	tgg	ctg	gtg	gtg	ctg	ctg	2231
Gly	Cys	Leu	Arg	Gly	Pro	Trp	Ala	Trp	Leu	Val	Val	Leu	Leu	
				145					150					

gcc	atg	ctg	gtg	gag	gtc	gca	ctg	tgc	acc	tgg	tac	ctg	gtg	2273

7

Ala 155	Met	Leu	Val	Glu	Val 160	Ala	Leu	Cys	Thr	Trp 165	Tyr	Leu	Val	
gcc Ala	ttc Phe 170	ccg Pro	ccg Pro	gag Glu	gtg Val	gtg Val 175	acg Thr	gac Asp	tgg Trp	cac His	atg Met 180	ctg Leu	ccc Pro	2315
acg Thr	gag Glu	gcg Ala 185	ctg Leu	gtg Val	cac His	tgc Cys	cgc Arg 190	aca Thr	cgc Arg	tcc Ser	tgg Trp	gtc Val 195	agc Ser	2357
ttc Phe	ggc Gly	cta Leu	gcg Ala 200	cac His	gcc Ala	acc Thr	aat Asn	gcc Ala 205	acg Thr	ctg Leu	gcc Ala	ttt Phe	ctc Leu 210	2399
tgc Cys	ttc Phe	ctg Leu	ggc Gly	act Thr 215	ttc Phe	ctg Leu	gtg Val	cgg Arg	agc Ser 220	cag Gln	ccg Pro	ggc Gly	cgc Arg	2441
tac Tyr 225	aac Asn	cgt Arg	gcc Ala	cgt Arg	ggc Gly 230	ctc Leu	acc Thr	ttt Phe	gcc Ala	atg Met 235	ctg Leu	gcc Ala	tac Tyr	2483
ttc Phe	atc Ile 240	acc Thr	tgg Trp	gtc Val	tcc Ser	ttt Phe 245	gtg Val	ccc Pro	ctc Leu	ctg Leu	gcc Ala 250	aat Asn	gtg Val	2525
cag Gln	gtg Val	gtc Val 255	ctc Leu	agg Arg	ccc Pro	gcc Ala	gtg Val 260	cag Gln	atg Met	ggc Gly	gcc Ala	ctc Leu 265	ctg Leu	2567
ctc Leu	tgt Cys	gtc Val	ctg Leu 270	ggc Gly	atc Ile	ctg Leu	gct Ala	gcc Ala 275	ttc Phe	cac His	ctg Leu	ccc Pro	agg Arg 280	2609
tgt Cys	tac Tyr	ctg Leu	ctc Leu	atg Met 285	cgg Arg	cag Gln	cca Pro	ggg Gly	ctc Leu 290	aac Asn	acc Thr	ccc Pro	gag Glu	2651
ttc Phe 295	ttc Phe	ctg Leu	gga Gly	ggg Gly	ggc Gly 300	cct Pro	ggg Gly	gat Asp	gcc Ala	caa Gln 305	ggc Gly	cag Gln	aat Asn	2693

8

gac	ggg	aac	aca	gga	aat	cag	ggg	aaa	cat	gag	tga	2729
Asp	Gly	Asn	Thr	Gly	Asn	Gln	Gly	Lys	His	Glu	*	
	310					315						

<210> SEQ ID No.: 7

<211> Length: 852

<212> Type: PRT

<213> Homo sapiens

<400> 7

Met	Leu	Gly	Pro	Ala	Val	Leu	Gly	Leu	Ser	Leu	Trp	Ala	Leu
1				5					10				

Leu	His	Pro	Gly	Thr	Gly	Ala	Pro	Leu	Cys	Leu	Ser	Gln	Gln
15					20					25			

Leu	Arg	Met	Lys	Gly	Asp	Tyr	Val	Leu	Gly	Gly	Leu	Phe	Pro
	30					35					40		

Leu	Gly	Glu	Ala	Glu	Glu	Ala	Gly	Leu	Arg	Ser	Arg	Thr	Arg
		45					50					55	

Pro	Ser	Ser	Pro	Val	Cys	Thr	Arg	Phe	Ser	Ser	Asn	Gly	Leu
			60					65					70

Leu	Trp	Ala	Leu	Ala	Met	Lys	Met	Ala	Val	Glu	Glu	Ile	Asn
				75					80				

Asn	Lys	Ser	Asp	Leu	Leu	Pro	Gly	Leu	Arg	Leu	Gly	Tyr	Asp
85					90					95			

Leu	Phe	Asp	Thr	Cys	Ser	Glu	Pro	Val	Val	Ala	Met	Lys	Pro
	100					105					110		

Ser	Leu	Met	Phe	Leu	Ala	Lys	Ala	Gly	Ser	Arg	Asp	Ile	Ala
		115					120					125	

Ala	Tyr	Cys	Asn	Tyr	Thr	Gln	Tyr	Gln	Pro	Arg	Val	Leu	Ala
			130					135					140

Val	Ile	Gly	Pro	His	Ser	Ser	Glu	Leu	Ala	Met	Val	Thr	Gly
				145					150				

9

Lys   Phe   Phe   Ser   Phe   Phe   Leu   Met   Pro   Gln   Val   Ser   Tyr   Gly
155   160   165

Ala   Ser   Met   Glu   Leu   Leu   Ser   Ala   Arg   Glu   Thr   Phe   Pro   Ser
     170   175   180

Phe   Phe   Arg   Thr   Val   Pro   Ser   Asp   Arg   Val   Gln   Leu   Thr   Ala
         185   190   195

Ala   Ala   Glu   Leu   Ser   Gln   Glu   Phe   Gly   Trp   Asn   Trp   Val   Ala
             200   205   210

Ala   Leu   Gly   Ser   Asp   Asp   Glu   Tyr   Gly   Arg   Gln   Gly   Leu   Ser
                215   220

Ile   Phe   Ser   Ala   Leu   Ala   Ala   Ala   Arg   Gly   Ile   Cys   Ile   Ala
225   230   235

His   Glu   Gly   Leu   Val   Pro   Leu   Pro   Arg   Ala   Asp   Asp   Ser   Arg
     240   245   250

Leu   Gly   Lys   Val   Gln   Asp   Val   Leu   His   Gln   Val   Asn   Gln   Ser
         255   260   265

Ser   Val   Gln   Val   Val   Leu   Leu   Phe   Ala   Ser   Val   His   Ala   Ala
         270   275   280

His   Ala   Leu   Phe   Asn   Tyr   Ser   Ile   Ser   Ser   Arg   Leu   Ser   Pro
            285   290

Lys   Val   Trp   Val   Ala   Ser   Glu   Ala   Trp   Leu   Thr   Ser   Asp   Leu
295   300   305

Val   Met   Gly   Leu   Pro   Gly   Met   Ala   Gln   Met   Gly   Thr   Val   Leu
    310   315   320

Gly   Phe   Leu   Gln   Arg   Gly   Ala   Gln   Leu   His   Glu   Phe   Pro   Gln
        325   330   335

Tyr   Val   Lys   Thr   His   Leu   Ala   Leu   Ala   Thr   Asp   Pro   Ala   Phe
        340   345   350

Cys   Ser   Ala   Leu   Gly   Glu   Arg   Glu   Gln   Gly   Leu   Glu   Glu   Asp
         355   360

10

Val Val Gly Gln Arg Cys Pro Gln Cys Asp Cys Ile Thr Leu
365   370   375

Gln Asn Val Ser Ala Gly Leu Asn His His Gln Thr Phe Ser
    380   385   390

Val Tyr Ala Ala Val Tyr Ser Val Ala Gln Ala Leu His Asn
        395   400   405

Thr Leu Gln Cys Asn Ala Ser Gly Cys Pro Ala Gln Asp Pro
            410   415   420

Val Lys Pro Trp Gln Leu Leu Glu Asn Met Tyr Asn Leu Thr
                425   430

Phe His Val Gly Gly Leu Pro Leu Arg Phe Asp Ser Ser Gly
435   440   445

Asn Val Asp Met Glu Tyr Asp Leu Lys Leu Trp Val Trp Gln
    450   455   460

Gly Ser Val Pro Arg Leu His Asp Val Gly Arg Phe Asn Gly
        465   470   475

Ser Leu Arg Thr Glu Arg Leu Lys Ile Arg Trp His Thr Ser
            480   485   490

Asp Asn Gln Lys Pro Val Ser Arg Cys Ser Arg Gln Cys Gln
            495   500

Glu Gly Gln Val Arg Arg Val Lys Gly Phe His Ser Cys Cys
505   510   515

Tyr Asp Cys Val Asp Cys Glu Ala Gly Ser Tyr Arg Gln Asn
    520   525   530

Pro Asp Asp Ile Ala Cys Thr Phe Cys Gly Gln Asp Glu Trp
        535   540   545

Ser Pro Glu Arg Ser Thr Arg Cys Phe Arg Arg Arg Ser Arg
            550   555   560

Phe Leu Ala Trp Gly Glu Pro Ala Val Leu Leu Leu Leu Leu
                565   570

Leu Leu Ser Leu Ala Leu Gly Leu Val Leu Ala Ala Leu Gly
575   580   585

11

Leu Phe Val His His Arg Asp Ser Pro Leu Val Gln Ala Ser
     590   595   600

Gly Gly Pro Leu Ala Cys Phe Gly Leu Val Cys Leu Gly Leu
          605   610   615

Val Cys Leu Ser Val Leu Leu Phe Pro Gly Gln Pro Ser Pro
               620   625   630

Ala Arg Cys Leu Ala Gln Gln Pro Leu Ser His Leu Pro Leu
                    635   640

Thr Gly Cys Leu Ser Thr Leu Phe Leu Gln Ala Ala Glu Ile
645   650   655

Phe Val Glu Ser Glu Leu Pro Leu Ser Trp Ala Asp Arg Leu
     660   665   670

Ser Gly Cys Leu Arg Gly Pro Trp Ala Trp Leu Val Val Leu
          675   680   685

Leu Ala Met Leu Val Glu Val Ala Leu Cys Thr Trp Tyr Leu
               690   695   700

Val Ala Phe Pro Pro Glu Val Val Thr Asp Trp His Met Leu
                    705   710

Pro Thr Glu Ala Leu Val His Cys Arg Thr Arg Ser Trp Val
715   720   725

Ser Phe Gly Leu Ala His Ala Thr Asn Ala Thr Leu Ala Phe
     730   735   740

Leu Cys Phe Leu Gly Thr Phe Leu Val Arg Ser Gln Pro Gly
          745   750   755

Arg Tyr Asn Arg Ala Arg Gly Leu Thr Phe Ala Met Leu Ala
               760   765   770

Tyr Phe Ile Thr Trp Val Ser Phe Val Pro Leu Leu Ala Asn
               775   780

Val Gln Val Val Leu Arg Pro Ala Val Gln Met Gly Ala Leu
785   790   795

12

Leu	Leu 800	Cys	Val	Leu	Gly	Ile 805	Leu	Ala	Ala	Phe	His 810	Leu	Pro
Arg	Cys	Tyr 815	Leu	Leu	Met	Arg	Gln 820	Pro	Gly	Leu	Asn	Thr 825	Pro
Glu	Phe	Phe	Leu 830	Gly	Gly	Gly	Pro	Gly 835	Asp	Ala	Gln	Gly	Gln 840
Asn	Asp	Gly	Asn	Thr 845	Gly	Asn	Gln	Gly	Lys 850	His	Glu	*	

13